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Review

Medial preoptic circuits governing instinctive social behaviors

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SUMMARY

The medial preoptic area (MPOA) has long been implicated in maternal and male sexual behavior. Modern neuroscience methods have begun to reveal the cellular networks responsible, while also implicating the MPOA in other social behaviors, affiliative social touch, and aggression. The social interactions rely on input from conspecifics whose most important modalities in rodents are olfaction and somatosensation. These inputs bypass the cerebral cortex to reach the MPOA to influence the social function. Hormonal inputs also directly act on MPOA neurons. In turn, the MPOA controls social responses via various projections for reward and motor output. The MPOA thus emerges as one of the major brain centers for instinctive social behavior. While key elements of MPOA circuits have been identified, a synthesis of these new data is now provided for further studies to reveal the mechanisms by which the area controls social interactions.

INTRODUCTION

Social behaviors play a crucial role in shaping how individuals interact with others of the same species. It is widely acknowledged that these behaviors have an instinctive component that is largely innate and characteristic of all members of the species and can be performed without any formal training.[1] In exchange, instinctive responses are fast and efficient. They are typically influenced by an individual's hormonal status and triggered by sensory cues. However, the animals exhibit a limited behavioral repertoire, largely determined by hard wiring. Therefore, instinctive behaviors are not as flexible and responsive to previous environmental influences as cortically driven cognitive and emotional responses, even though early life stressors may alter instinctive social behaviors.[2] Furthermore, the internal state of the animal can also modify the execution and neuronal circuits of these behaviors.[3] The control of instinctive social behaviors involves several subcortical brain regions.[4] This article emphasizes the crucial role played by the preoptic area (POA), specifically the medial preoptic area (MPOA) in these regulatory actions. The process of reproduction involves not only hormone-driven physiological processes but also critically important instinctive behavioral components, which are the subject of the article. The review will cover other types of social behaviors, too, such as allogrooming and other affiliative behaviors, as well as antagonistic behaviors, including aggression, which are also controlled by preoptic circuits.

The hypothalamus plays a vital role in maintaining homeostasis in the body by regulating the hormonal status of animals as well as their autonomic functions. It is beneficial to regulate instinctive social behaviors in synchrony with maintaining the body's general homeostasis. This can be achieved by the MPOA, a brain region that is closely connected to the endocrine and autonomic centers. Although the MPOA plays a crucial role in controlling social behaviors, there has been no specific review on this topic. The closest recent reviews on the subject do not focus on the MPOA,[4,5] while recent reviews on the POA did not address its role in controlling social behaviors.[6–8] In this review, we first discuss recent developments in identifying the cell types of the POA. We then examine the preoptic circuits that govern reproductive, affiliative, and aggressive behaviors in experimental rodent species. Additionally, we focus on the hormonal and neuronal inputs that affect the preoptic neurons involved in controlling various social behaviors. Finally, it is discussed how the instinctive behaviors governed by the MPOA relate to cortical control of social behaviors.

STRUCTURE AND CELL TYPES OF THE PREOPTIC AREA

The POA is located in the most anterior region of the hypothalamus. It is named after its location, which is immediately anterior to the optic chiasm, where optic nerve fibers partially cross the midline and enter the brain from a ventral direction.[9] Mediolaterally, it can be divided into three zones: periventricular, medial, and lateral, which consist of different areas and nuclei (Figure 1).

The POA contains only a few nuclei including the median preoptic nucleus devoted to the control of body temperature,[7] and the ventrolateral preoptic nucleus devoted to sleep regulation.[6,13,14] The present review will not discuss these nuclei as they are not known to have a direct role in regulating social behaviors. However, the anteroventral periventricular (AVPV), medial preoptic nucleus (MPN),

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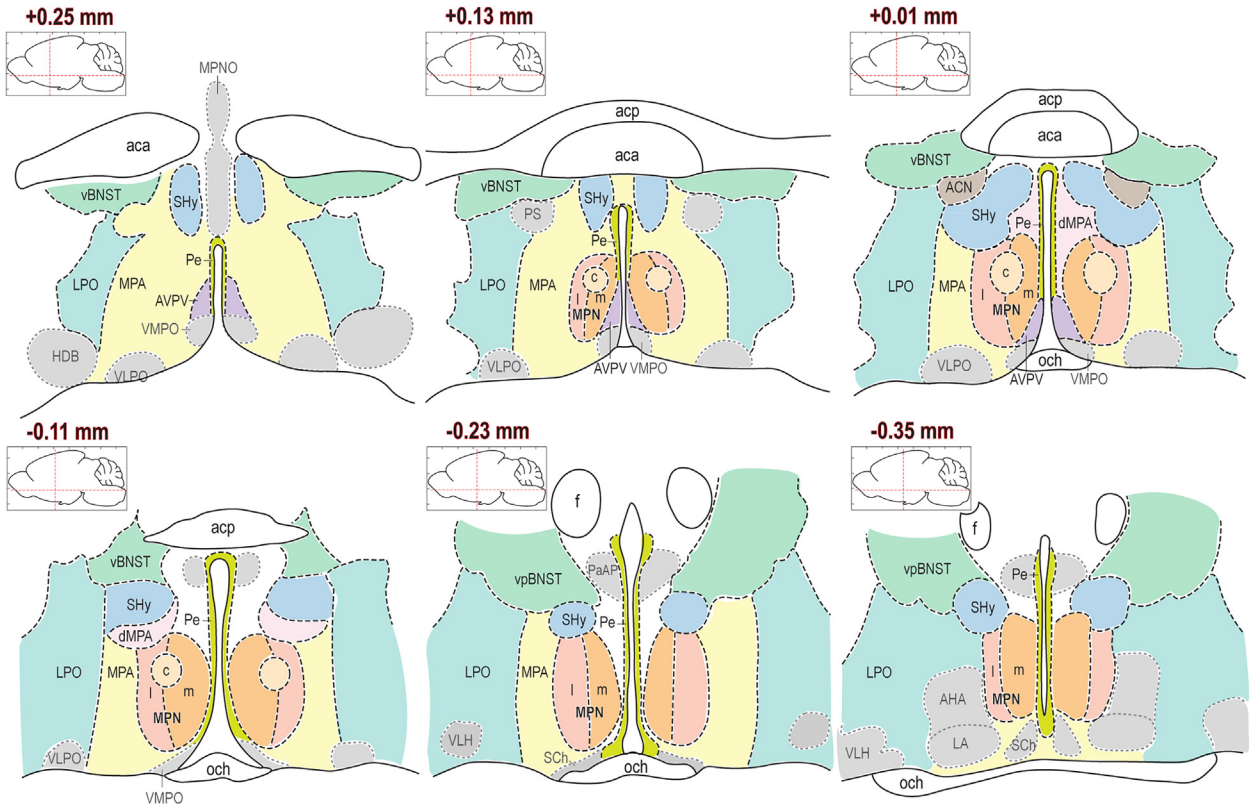

Figure 1. Schematic diagram shows the preoptic area (POA) and its different subdivisions

The POA is located adjacent to the third ventricle. Its periventricular zone contains the periventricular hypothalamic (Pe) and the anteroventral periventricular (AVPV) nuclei. The medial zone includes the medial preoptic nucleus (MPN), the surrounding medial preoptic area (MPOA), the septohypothalamic (SHy), and anterior commissural nuclei (ACN). Functionally, the ventral subdivision of the bed nucleus of stria terminalis (vBNST), which is situated dorsally, is also classified within the medial zone. The MPN and MPA can be further divided into subgroups. The MPN is divided into medial (m), central (c), and lateral (L) regions, while the MPA includes a dorsal area (dMPA). The illustration is based on Paxinos and Franklin's The Mouse Brain in Stereotaxic Coordinates,[10] and is aligned with a publication on the cellular composition of the preoptic area.[11] The delineation of the medial zone is adapted from a study focusing on this area.[12] Further abbreviations: aca: anterior commissure (anterior part), acp: anterior commissure (posterior), AHA: anterior hypothalamic nucleus (anterior), HDB: nucleus of the horizontal limb of the diagonal band, LA: lateral amygdaloid nucleus, MnPO: median preoptic nucleus, PaAP: paraventricular hypothalamic nucleus (posterior part), PS: parastrial nucleus, SCh: suprachiasmatic nucleus, VLH: ventrolateral hypothalamic nucleus, VLPO: ventrolateral preoptic nucleus, VMPO: ventromedial preoptic nucleus.

septohypothalamic (SHy), and anterior commissural nuclei (ACN) are involved in controlling social behaviors. Additionally, the area surrounding the MPN in the medial zone of the preoptic region, known as the MPOA, also plays a crucial role in controlling social behavior. No nuclei can be distinguished within the MPA, so it remains an area instead of being called a nucleus. The MPA is contiguous with the ventral subdivision of the bed nucleus of the stria terminalis (vBNST) dorsally. Although the vBNST and MPA are discrete anatomical structures, they are intimately connected through neural pathways that facilitate the collaborative regulation of a diverse array of behavioral and physiological functions, particularly those related to reproduction, stress responses, and social interactions. As in the medial zone, the nuclei within the lateral POA are not readily distinguishable from one another, with the exception of the ventrolateral preoptic nucleus. Consequently, the lateral zone can be designated as the lateral POA. Although no nuclei can be delineated in these parts of the POA, some dispersed neuronal groups functionally belong together. These include the gonadotropin-releasing hormone-containing neurons, which project to the median eminence and control luteinizing hormone and follicle-stimulating hormone secretion from the pituitary,[15] as well as the warmth-sensitive neurons in the MPA controlling body temperature.[7,14] As these well-defined cell groups do not directly control social behavior, they will not be discussed further in this review. The involvement of other neurons in the MPOA in controlling social behavior was initially demonstrated through c-Fos activation studies. A high density of c-Fos-positive neurons appears in the MPOA in response to conspecifics, indicating intense activation of these neurons. However, neither the development, nor the molecular characterization of these neurons and their specific functions has yet to be established. Recently, advanced methods were used to characterize the socially engaged neurons of the POA, making it one of the most well-characterized brain regions and a model for other brain areas and functions. A recent publication addresses a long-standing question regarding the temporal and spatial development of the preoptic cell population and the factors influencing it. This study offers new insights into POA development and contributes to an understanding critical periods in the development and function of instinctive

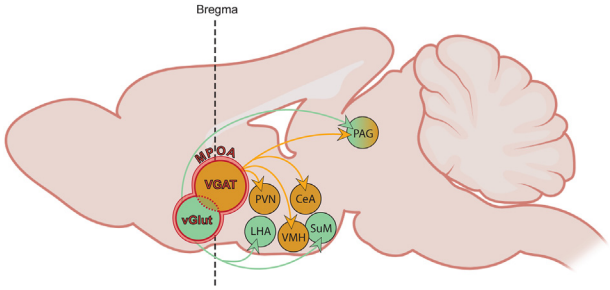

Figure 2. A schematic of the parasagittal rodent brain showing the primary projections of glutamatergic and GABAergic preoptic neurons

The dashed line indicates the position of bregma. In the preoptic area, vesicular glutamate transporter 2-positive (VGlut2+) glutamatergic neurons are marked by a bold red border on a light green background. Their primary projection sites are denoted by green arrows and light green circles outlined in black. Vesicular GABA transporter-positive (VGAT+) GABAergic neurons in the preoptic region are distinguished by a bold red border and orange coloration. Their projections are indicated by orange arrows and orange circles outlined in black. While vGlut2+ and VGAT+ neurons comprise essentially all preoptic neurons, some cells express both markers.[11] The region enclosed by the dotted line indicates the overlapping population of preoptic vGlut2+ and VGAT+ cells. The major projections of vGlut2+ and VGAT+ neurons include all major projections of the POA. However, distinct cell groups within the POA can have unique projections. Areas with overlapping projections are marked with a gradient. Further abbreviations: AVPV: anteroventral periventricular nucleus, DM: dorsomedial hypothalamic nucleus, LHA: lateral hypothalamic area, PAG: periaqueductal gray, PVN: paraventricular hypothalamic nucleus, SuM: superior mammillary nucleus, VMH: ventromedial hypothalamic nucleus.

behavior circuits during early life. By employing paired single-nucleus gene expression and chromatin accessibility sequencing (snRNA-seq and snATAC-seq) techniques, researchers were able to identify cell types associated with homeostatic or social behavior functions and characterize diverse maturational trajectories influenced by POA sub-region, behavioral function, sex and the presence of sensory input from specific modalities, such as somatosensation and socially relevant chemosensation.[16] Furthermore, another state-of-the-art, spatially resolved single-cell sequencing technique called MERFISH was used to identify the cell types in the area, and hybridization methods were used to identify the position of the different cell types based on selected marker genes.[11] While it is reasonable to assume that cell types belonging to a gene expressional cluster have the same functions, it was confirmed that neuronal cell types clustered based on their similar gene expression do not always cluster in space. The study identified a total of 43 inhibitory, 23 excitatory, and 3 neuronal cell types that demonstrated both glutamate and GABAergic phenotype. This extensive database enables the identification of projections and inputs in the POA of each cell type in the future. In addition, it provides genes that are expressed in a cell-type-specific manner. These genes can be used to develop mouse lines for selectively manipulating the neuronal activities of different neurons and measuring their activities in relation to animal behavior. For example, Cre recombinase can be expressed using the promoter of cell-type-selectively expressed genes. In addition, it became possible to identify all cells *in vivo* for their electrophysiological characterization by using selectively expressed fluorescent marker proteins. Previous studies have established projection outputs from GABAergic and glutamatergic MPOA subpopulations (Figure 2). However, projections from the genetically-defined subclusters identified by Moffitt and colleagues have not yet been reported but will be technically possible in the future. The different cell types are likely to have distinct projections as has been demonstrated for preoptic GABAergic and glutamatergic neurons. GABAergic neurons have major projections to the paraventricular hypothalamic nucleus (PVN) and surrounding area, the ventromedial hypothalamic nucleus (VMH), the periaqueductal gray (PAG), and the central amygdaloid nucleus.[17] The glutamatergic neurons send their densest projections to the PAG, the lateral hypothalamic area, and the supramammillary nucleus, which may mediate different parenting actions. Additionally, GABAergic neurons can suppress glutamatergic neurons within the POA, indicating local regulation, too.[18]

CIRCUITS FOR THE DIFFERENT SOCIAL BEHAVIORS IN RODENTS

Rodent species commonly used as model animals in laboratories are social, allowing for the study of their social behaviors under experimental conditions. The high level of sociability exhibited by rodents can be quantified and validated through various laboratory experiments. These experiments demonstrate that the test subjects consistently exhibit a preference for being with conspecifics over empty or non-social environments.[19,20] This preference is driven by the presence of conspecifics by resulting in heightened midbrain dopaminergic activity as a reward during social interactions.[21,22] Animal social behaviors can be classified into three groups: reproductive (parental and sexual), affiliative or cooperative (such as allogrooming and social play), and competitive, including aggressive behaviors. The occurrence of these behaviors depends on the animals and their condition. Sexual behavior in female rodents only occurs during estrus, which lasts for about a day in a five-day cycle. Caring behavior is also dependent on the situation, occurring when pups are given to dams. Aggressive behavior may occur between two males or when an intruder is placed in an animal's own cage. A mother may also aggressively defend her pups. However, if the animals are familiar with each other or two females are tested in a large enough cage, aggressive behavior is rarely observed. During a typical 10-min test period, the animals are active for approximately half of the time, with a greater portion of their active time devoted to affiliative social behavior compared to non-social behavior.[23] All types of social behaviors are greatly reduced in anxious animals, therefore it is important to ensure that animals are not stressed during behavioral testing.[18]

The research on the role of the MPOA in different types of social behaviors is still in its early stages. Still, understanding the cell types involved in the regulation of instinctive behaviors controlled by the POA and their neuronal connections and functions, as discussed below, is a crucial step. The critically important core information is also summarized in Table 1. The review discussed four major types of social behaviors, which are controlled by the POA. These include parental behavior, male sexual behavior, affiliative social behavior, and aggression (Box 1). Female sexual behavior is not discussed in detail because that behavior is predominantly driven by the ventromedial hypothalamic nucleus. However, the POA also plays a role in the control of female sexual behavior, and we refer to specific reviews for further reading.[24,25]

Parenting behavior

Parenting behaviors can be categorized based on whether they are directed toward the offspring. In laboratory rodent species, the most significant pup-directed behaviors include nursing, anogenital licking, and retrieving the pups to the nest. Nest building and nest defense are examples of non-pup directed behavior.[45] The latter one also demonstrated an increased risk-taking behavior on the part of the mother. When a rodent mother perceives a threat to her nest, such as the presence of a predator or intruder, she may engage in aggressive behaviors to defend her pups. These behaviors may include vocalizations, charging at the threat, and even physical attacks. The significance of the MPOA in the aforementioned behaviors is underscored by the observation that its lesion selectively abolishes both direct and indirect elements of parenting behaviors.[46–49] In addition, MPOA neurons are activated in response to pup exposure as detected by the appearance of c-Fos immunolabelled neurons[50–53] (Figure 3). It is likely that the elimination of these neurons was important in the lesion studies for the cessation of maternal behavior.

Traditional histological techniques have established that the neurons activated in mothers in response to pups are predominantly GABAergic inhibitory cells.[54] This was confirmed by single-cell sequencing in an approach, which utilized that the elevated neuronal activity can be assessed from the amount of c-Fos mRNA.[11] This approach also allowed the determination of the dominant expression of a single GABAergic cell cluster in virgin females in response to pup exposure. The pup-induced activation of neurons belonging to this cluster was also dominant in mothers and fathers.

The significance of preoptic GABAergic neurons in controlling maternal behaviors was demonstrated when chemogenetic[17] and optogenetic[18] stimulation promoted maternal behaviors in females while their inhibition reduced them. In turn, optogenetic inhibition of preoptic glutamatergic neurons enhanced parenting.[18] The data indicate that preoptic GABAergic neurons facilitate parenting in females, while glutamatergic neurons inhibit it. In addition to investigating GABA and glutamatergic neurons, other subpopulations of preoptic neurons have also been studied for their involvement in parenting. Optogenetic activation of galanin-containing preoptic neurons has been found to promote parental behaviors (Figure 5). Conversely, the elimination of galanin neurons inhibited parenting, as the number of surviving galanin neurons was reduced by ablation.[28] It has also been demonstrated that the majority of galanin neurons are c-Fos-activated following pup exposure.[28,29] Subsequently, it was discovered that galanin is expressed in five different inhibitory and two excitatory cell types in the POA.[11] However, the highest expression was found in an inhibitory neuronal cluster that was activated by pup exposure in virgin females. This suggests that the stimulation and ablation of these galanin-positive cells may have contributed the most to the parental behavioral effects. The preoptic galanin neurons primarily target the AVPV, PVN, and dorsomedial hypothalamic nucleus (Figure 2). Additionally, they also provide input to the ventral tegmental area (VTA) and MeA, albeit to a lesser extent.[31] When functions of different projections were addressed with optogenetics, the projection to the PAG increased pup grooming and pup-directed sniffing bouts. In contrast, the projection to the MeA did not affect pup behavior.[31] The projection to the VTA increased barrier crossing toward the pups, indicating elevated maternal motivation. This is consistent with maternal behavior being a robust and rewarding motivated behavior.[22] The VTA projections likely terminate on VTA GABAergic and not dopaminergic neurons[55] suggesting that the ascending dopaminergic reward pathway is activated by disinhibition.

Dopaminergic neurons in the AVPV have also been shown to control maternal behavior.[32] Optogenetic stimulation of these cells increased maternal behaviors, while their chemical ablation reduced it in females. The number of these cells was higher in females than in males. Parenting was not affected by the manipulation of these cells in virgin males and fathers. This suggests a sexual dimorphism regarding this cell type, which may form the basis of the difference between maternal and paternal care behavior. The overexpression of tyrosine hydroxylase, which is the rate-limiting enzyme of dopamine synthesis, in these neurons, imitated the effects of stimulation. This suggests that the dopamine content of these neurons, as a neurotransmitter, was involved in the action of the cells.[32] In addition to dopamine, these cells may possess GABA as well as galanin[11] and therefore, may contribute to the difference in behavior between females and males governed by the manipulation of galanin neurons.[28] It was also demonstrated that these AVPV dopaminergic neurons project to oxytocin neurons of the PVN and elevate their oxytocin secretion.[32] These findings indicate that dopaminergic neurons may influence maternal behaviors by activating oxytocin neurons. However, other potential mechanisms, such as the role of additional projections of AVPV dopaminergic neurons, or neuroplastic, or epigenetic alterations in maternal centers cannot be ruled out, either.[56]

Amylin-expressing neurons and neurons expressing the receptor of amylin, the calcitonin receptor (Calcr), are additional preoptic cell groups implicated in the control of maternal behavior. The neuropeptide amylin was first identified in the brain as the gene demonstrating the highest increase in its expression in the POA of mother rats as compared to non-maternal animals.[57,58] Amylin is also induced by maternal sensitization in female rats, in neurons, which are c-Fos activated following pup exposure.[58] Knockdown of amylin gene expression impeded risk-taking maternal care (e.g., retrieving pups from the open arm of an elevated plus maze), and specific silencing of its receptor in the MPOA inhibited nurturing behaviors.[33] This suggests that the amylin-Calcr neuromodulatory system may play a role in promoting maternal behavior. This is further corroborated by the observation that not only amylin but also Calcr is upregulated in mother animals, particularly in the preoptic GABAergic cells. Consequently, the distribution of Calcr is altered in mothers because Calcr is predominantly expressed in glutamatergic

Table 1. The neuronal connections of different cell types in various preoptic subregions and the behavioral effects of their manipulations

MPOA sub-region	Cell type	Type of manipulation	Behavioral effect	Input	Output	References
Parenting behavior						
MPOA	GABAergic neurons	chemogenetic stimulation chemogenetic inhibition optogenetic stimulation optogenetic inhibition	promoted maternal behaviors in females reduced maternal behaviors in females increased infanticide behavior in male mice reduced infanticide behavior in male mice	PIL	PVN, Spa, PAG, VMH, CeA, SON, LH, VTA	Dimen et al.[17] Keller et al.[26] Zhang et al.[18]
MPOA	Glutamatergic neurons	optogenetic inhibition	inhibition enhanced parenting in females	LS, BNST, PVN, VTA	PAG, LH, SuM	Zhang et al.[18] Smith et al.[27]
MPOA	Galanin-containing preoptic neurons	optogenetic stimulation elimination of galanin neurons	promoted parental behaviors reduced infanticide behavior in virgin male mice inhibited parenting increased pup grooming and pup-directed sniffing bouts increased barrier crossing toward the pups, indicating elevated maternal motivation	NAc, NAsh, AVPe, VOL, MnPO, VMPC, MS, IL, LS, BNST, PVT, PVN, AH, MeA, BMA, VMH, PIL, SON, DM, PMV, AHPM, RM, VTA, SNpc, PAG, LC, RMg	AVPV, NAc, LS, BNST, PVN, PVT, SFO, PeFA, MeA, SON, Arc, VMH, DM, RM, VTA, SNpc, RMg, RRF, PAG, LC	Wu et al.[28] Cservenak et al.[29,30] Kohl et al.[31]
AVPV	Dopaminergic neurons	optogenetic stimulation and overexpression of tyrosine hydroxylase chemical ablation	increased maternal behaviors reduced maternal behaviors in females paternal behavior was not affected in virgin males and fathers	–	LS, BNST, MPOA, PVN, SON, Arc, LH, DM	Scott et al.[32]
cMPOA and ACN	Amylin-expressing neurons	knockdown of amylin gene expression	impeded risk-taking maternal care	–	–	Yoshihara et al.[33]
	Calcitonin receptor expressing neurons	silencing of calcitonin receptor chemogenetic stimulation of calcitonin receptor	inhibited nurturing behaviors inhibited infanticide in virgin males	MPOA, BNST, PVN, AHA, VMH, Arc, LSv, LSi, LH	LSv, Acb, BNST, MeA, AHA, SON, PVN, Pe, RCH, DM, VMH, PAG, VTA, SNc, A8 DA cells, LC	Yoshihara et al.[33]
vBNST	ERα-expressing neurons	optogenetic activation chemogenetic inhibition	induced infanticide in female mice promoted maternal behavior in virgin females and mothers	MPOA	MPOA	Mei et al.[5,34,35]
MPOA	ERα-expressing neurons	ablation chemogenetic inhibition chemogenetic inhibition optogenetic stimulation optogenetic stimulation	induced infanticide in virgin female mice inhibited pup retrieval in mother mice promoted pup approach and retrieval promoted pup approach and retrieval	BNST, VMH, SUM, PVN, PVt, PMv, NAc, LS, AVPV, AHN, MeApd, DMSH, VTA, PAG	BNST, PVN, Pv, Arc, RCh, DMH, VMHvl, Tu, LHA, PMv, SUM, PAG, VTA	Mei et al.[5,34,35] Fang et al.[36]

(*Continued on next page*)

Table 1. *Continued*

MPOA sub-region	Cell type	Type of manipulation	Behavioral effect	Input	Output	References
male sexual behavior						
MPOA	ERα-expressing neurons ERα-expressing GABAergic neurons Tac1-expressing neurons	chemogenetic inhibition optogenetic stimulation optogenetic stimulation optogenetic activation optogenetic inhibition	decreased mounting toward females promoted female-directed mounting as well as USV converted male-directed attack to mounting with USVs reduced mating latency suppressed aggression increased mounting abrogated mating	VMH BNST	VTA PAG	Karigo et al.[37] Bayless et al.[38,39]
vBNST	Tac1/ERα neurons	chemogenetic inhibition	eliminated sex recognition, reduced mounting during mating, and attacks during aggression in males	–	–	Knoedler et al.[40]
MPOA	Tac1-expressing neurons	optogenetic activation optogenetic inhibition	suppressed aggression and promoted mating with males without altering mating with females suppressed mating	–	MPOA	Bayless et al.[38,39]
MPOA	Neurotensin neurons	photostimulation	enhanced female preference and social interaction	–	VTA	McHenry et al.[41]
affiliative social behaviors						
MPOA and the ACN	Calcitonin receptor (Calcr) Amylin neurons	knockdown chemogenetic stimulation	reduced contact behaviors increased contact-seeking behavior	–	–	Fukumitsu et al.[42]
MPOA	Glutamatergic neurons expressing the melanocortin receptor 4 (MC4R)	optogenetic activation in mice housed in groups optogenetic inhibition	increased social interaction enhanced social preference conceived negative valence reduced social preference and rebound	reunion-induced MPN neurons, IL, LS, NAc, BNST, PVN, PVT, ZI, MeA, Arc, VMH, PMV, PBN, vCA1	LS, NAc, BNST, PVN, AH, Arc, VMH, SUM, PMV, LS, Hb	Liu et al.[43]
	inhibitory neurons expressing the Thyrotropin-releasing hormone receptor (Trhr)	optogenetic activation	decreased social rebound during social reunion conceived positive valence	NAc, AVPe, LS, BNST, PVN, Arc, VMH, PMV, MeA, CeA, PAG, VTA, LC, SUM, vCA1, PBN	NAc, LS, BNST, PVN, PVT, LH, VMH, Arc, MeA, PMV, SUM, VTA, PAG, DR, LC, PBN	Liu et al.[43]
	Neuro-tensin neurons	optogenetic stimulation	enhanced opposite-sex conspecifics preference in both sexes	–	VTA	McHenry et al.[41]
aggression						
MPOA	ERα-positive GABAerg neurons	optogenetic stimulation	reduced aggression, converted male-directed attack to mounting with ultrasonic vocalizations		VMHvl	Karigo et al.[37]
caudalMPOA	ERα-positive	chemogenetic inhibition chemogenetic stimulation	increased aggression suppressed intermale aggression		VMHvl	Wei et al.[44]

Box 1. The definitions and characteristics of social behaviors controlled by the preoptic area

Parental behavior

Definition:
Pup caring behavior refers to the actions of mothers who nurture and protect their offspring. This includes maternal aggression towards male intruders as a form of protection.

Behavioral elements:
Pup-directed behaviors (nursing, anogenital licking, and retrieving the pups to the nest) and non pup-directed behaviors (nest building, maternal aggression)

Method of measurement:
Testing the actions of mothers following the return of a litter that was previously separated for a brief period. The pup retrieval test measures the time it takes for the mother to retrieve three pups placed outside of the nest in the homecage or from an arm of a maze. Nest building can be evaluated by observing the destruction of the nest, while maternal aggression can be assessed by introducing a male intruder to the mother's homecage.

Male sexual behavior

Definition:
Male rodents engage in sexual behavior to inseminate a female

Behavioral elements:
Mounting, intromission, ejaculation, the emission of specific ultrasonic vocalization, as well as post-ejaculatory behaviors

Method of measurement:
The test of sexual behavior involves introducing a sexually receptive female (e.g. ovarectomized and estrogen primed) to a male animal and recording and analyzing their interactions.

Affiliative social behavior

Definition:
Social interaction between individuals with positive valence that do not involve aggression or sexual behavior

Behavioral elements:
Approaching, sniffing, head-to-head contact, crawling under and allogrooming

Method of measurement:
Direct social interaction between 2 individuals, often using adult female animals as subjects. The test is performed in a familiar environment but not the home cage of either animal. Previous social isolation is often necessary to immediately increase interaction.

Aggression

Definition:
Action aimed at threatening or harming others motivated by competition for resources, territory, or social status

Behavioral elements:
Aggressive mounting, pushing, and biting aimed at a conspecific

Method of measurements:
The intruder test is a method of measuring aggression, which involves introducing an unfamiliar animal into the home cage of a single-housed animal. Aggression is generally higher among males than females. Additionally, prolonged social isolation may be needed to increase aggressive behavior.

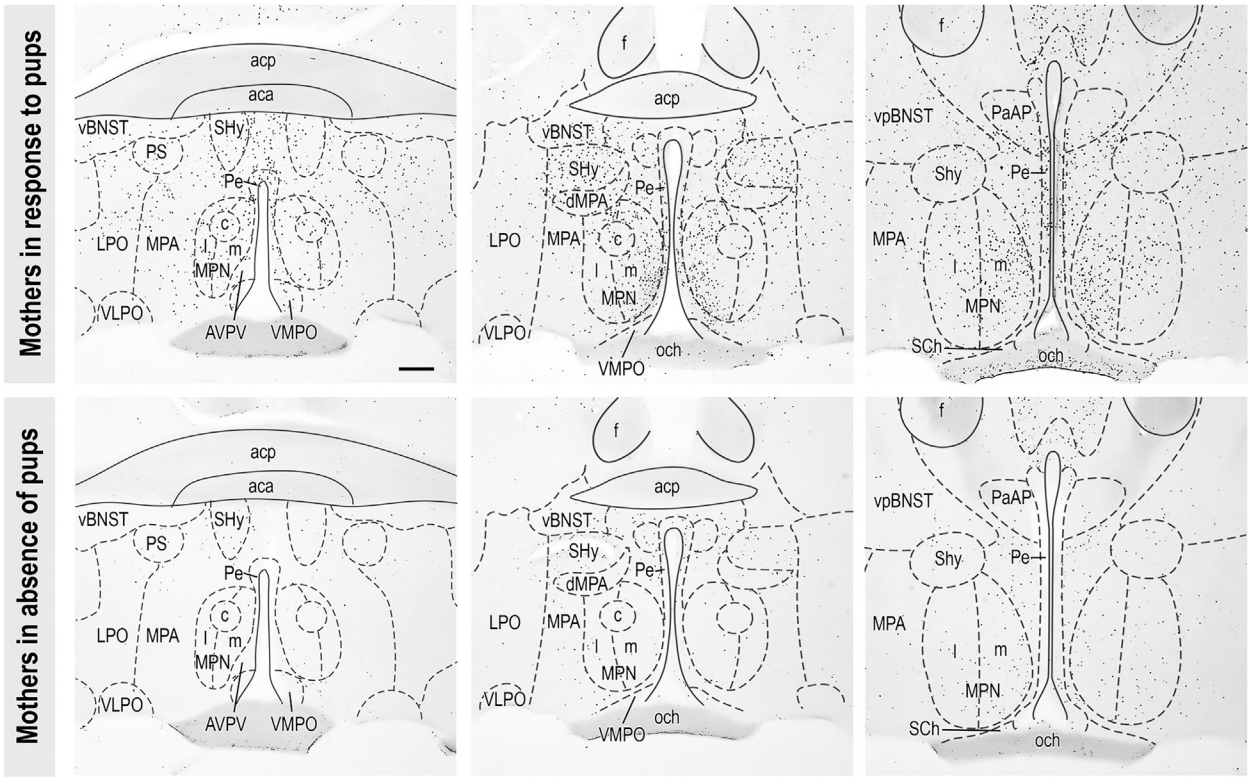

Figure 3. Neuronal activation in the preoptic area (POA) of mother mice after exposure to pups compared to that in mother mice separated from their pups

Mother mice after 22 h of pup separation with and without receiving their pups back. The coronal sections demonstrate three distinct anatomical levels. The upper panels display representative light microscopic images in a mother mouse demonstrating elevated expression of thec-Fos protein visualized by immunohistochemistry. The lower panels display representative images of a mother mouse separated from her pups, revealing a significantly lower number of activated neurons in the medial preoptic region (MPOA). The region of the medial preoptic nucleus (MPN) and the medial preoptic area (MPA) are bordered by dashed lines. The templates used to indicate the boundaries of the brain regions can be found in the legend of Figure 1. The abbreviations for additional anatomical regions can be found in the legend of Figure 1 and in the list of abbreviations. Scale bar: 200 μm.

neurons in sexually inexperienced females.[33] Additionally, it is expressed in galanin neurons that are activated by pups in mother mice[11] suggesting that amylin might exert its action via these preoptic cell types.

It is important to note that virgin males do not exhibit parenting behavior, but rather infanticide. The involvement of GABAergic neurons in the control of infanticide behavior is suggested by the fact that chemogenetic stimulation increased while inhibition reduced infanticide behavior in male mice.[17,18] It is possible that the opposite effect in males than in females could be due to an opposing role of the pup-activated GABAergic cluster or a dominant other GABAergic cell type promoting infanticide behavior in males. Activation of preoptic galanin neurons has been shown to reduce infanticide behavior in male mice,[28] indicating that non-galaninergic but GABAergic neuronal populations may drive infanticide behavior in virgin males. Infanticide can sometimes also occur in female mice. Estrogen receptor α (ERα)-expressing neurons located in the principal nucleus of the bed nucleus of stria terminalis play a crucial role in infanticide in female mice. These neurons are necessary, sufficient, and naturally activated during this behavior (Figure 5). Input to these cells from the ERα-expressing neurons of the MPOA inhibits this behavior[34] demonstrating a way how MPOA not only promotes parenting but also counteracts infanticide behavior. The involvement of preoptic ERα cells in caregiving behavior is demonstrated by their experimental inactivation, which abolishes pup approach and retrieval behavior. In contrast, optogenetic activation induces immediate pup retrieval behavior, primarily through its projection to the VTA.[36]

Male sexual behavior

Male sexual behavior in laboratory rodents includes approaching a receptive female, mounting, intromission (penetration), and ejaculation[59] as well as the emission of specific ultrasonic vocalization (USV),[60] followed by a refractory period. These behavioral elements are eliminated by lesions of the POA suggesting that sexual drive is regulated by this brain region.[46,61] Since a large number of neurons are activated in the MPOA following male sexual behavior,[62–66] it is reasonable to assume that the elimination of these neurons belonging to 5 inhibitory and 2 excitatory cell types, and barely overlapping with neurons activated by parenting,[11] plays a critical role in the effects of preoptic lesions.

In the MPOA, *in vivo*, calcium imaging at single-cell resolution has demonstrated neurons that preferentially respond to females.[67] These are likely predominantly ERα-expressing neurons.[37] Female-directed sniffing and mounting were represented by largely distinct groups of neurons.[67] The involvement of MPA ERα-positive cells in sexual behavior directed specifically toward females is substantiated by their heightened activity during mounting behavior, rather than during aggressive interactions with male conspecific. This indicates that preoptic ERα cells contribute to the network biased toward mating.[68] To further support, optogenetic stimulation of ERα-expressing GABAergic cells, but not all preoptic ERα neurons, induces female-directed mounting as well as USV characteristic of male sexual behavior.[37] In solitary males, it was still possible to evoke USV, indicating a direct role of these cells in USV as well. It was also shown that optogenetic activation of ERα-expressing VMH neurons projecting to the MPOA inhibited mounting behavior and USV[37] suggesting antagonistic intrahypothalamic control of the ERα-expressing GABAergic MPOA neurons.

The preoptic neurons expressing the tachykinin receptor 1 (Tacr1) have been identified as the cell group that is critically important for male sexual behavior (Figure 5). These cells are activated in males in response to anogenital sniffing of a female, mounting, and intromission, but not in response to sniffing or attacking another male. In addition, the activation of these cells through optogenetics reduced the time it took to mate and resulted in synchronized mating behavior toward a toy mouse, another male, or a non-receptive female in the absence of a receptive female partner.[38] To demonstrate the functional importance of Tacr1-expressing preoptic neurons in male sexual behavior, their optogenetic inhibition abrogated mating with females without altering sniffing. This finding was confirmed by chemogenetic inhibition of Tacr1-expressing preoptic neurons, which eliminated mating without affecting territorial aggression and other behaviors.[38] Additionally, Tacr1-expressing preoptic neurons activated the reward center and led to dopamine release in the nucleus accumbens. Furthermore, male mice demonstrated optogenetic self-stimulation of their Tacr1-expressing preoptic neurons. It was also found that the neuropeptide substance P potentiates the activation of Tacr1-expressing preoptic neurons in a Tacr1-dependent manner to initiate male sexual behavior. To gain a better understanding of the mechanisms of action of these neurons, their neuronal targets were investigated. These neurons project to various brain regions, including the PAG and the VTA. Activation of both projections elicited mounting. However, mounting induced by activation of VTA projections did not progress to intromissions, whereas activation of PAG projections led to mounting and intromission. This suggests that VTA projections are rewarding, while the PAG projections carry out the motor actions. These actions of Tacr1 neurons did not depend on whether the males were sexually experienced, indicating their innate property.[38] Neurotensin neurons are another group of preoptic neurons that convey the reward value of sexual activity to the VTA. When these neurons were photostimulated, female preference was enhanced, and the amount of time spent in female social interaction increased. Additionally, anterograde neuroanatomical tracing revealed that these cells project to the VTA, and photostimulation of these projections evoked dopamine release in the nucleus accumbens.[41]

Within the PAG, various cells may mediate mounting and USV. A USV-specific PAG neuron type has been identified, which is activated by preoptic inhibitory neurons through disinhibition via a local circuit inhibitory interneuron in the PAG as the direct target of the preoptic projection.[69]

Affiliative social behaviors

When animals are reunited after a short period of social isolation, they exhibit elevated affiliative behaviors while longer period of social isolation triggers aggressive and/or depressive behaviors in rodents.[70,71] The behavioral modules of affiliative behaviors include approaching, sniffing, crawling under, head-to-head contact, and allogrooming between two familiar individuals of the same sex, between which aggression and sexual behavior does not happen.[42,43] Although these sequences involve reciprocal interactions between two animals placed together, the behavior events are mostly initiated by the animal that had been previously isolated. The duration of interaction and the number of interaction bouts increase with increasing periods of preceding isolation. Somatic isolation - where the animals could not touch each other but were able to be in contact via other modalities - is sufficient to increase contact behavior.[42,43] During the reunion, the display of rebound behavior declines over time, indicating a gradual satiation of social drive.[43] Neurons participating in the homeostatic control of social interactions may have altered activity during social interactions, which indeed lead to an increased number of c-Fos-activated neurons in different parts of the POA, the MPN, the caudal part of the MPOA, and the ACN.[26,42] The activation of preoptic neurons during the reunion is crucial for the development of an appropriate behavioral response. This is evidenced by the cessation of reunion-induced social investigation and USV production upon the inhibition of reunion-responsive c-Fos-expressing neurons.[72] Some activated neurons in the MPOA and the ACN express the Calcr, which has been previously described to play a role in maternal behavior. In the MPOA, social interaction-induced c-Fos-positive neurons contained Calcr, while in the ACN, c-Fos-positive neurons were negative for Calcr. Most ACN Calcr neurons were GABAergic, while MPOA Calcr neurons were glutamatergic based on co-expression of specific markers.[42] Injection of amylin, the ligand of Calcr, induced c-Fos in Calcr neurons. Amylin neurons are also located in the MPOA.[57,58] Chemogenetic stimulation of amylin neurons increased contact-seeking behavior.[42] In turn, the knockdown of Calcr in the cMPOA using RNA interference reduced contact behaviors. In addition, amylin expression in the MPOA was reduced by social isolation and induced by the presence of conspecifics. Therefore, it is reasonable to assume that the amylin-Calcr neuromodulator system of the MPOA is involved in affiliative contact behaviors in addition to its above described role in maternal care.

In another study, c-Fos positive neurons induced by isolation and reunion were found to form two distinct cell populations in the MPN. This was achieved by using a combination of tamoxifen-inducible Cre line TRAP2, which labeled integrated activity across several hours during social isolation, and c-Fos *in situ* hybridization, which visualized transiently activated neurons during social rebound.[43] Upon further identification, it was discovered that the neurons induced by isolation belonged to the glutamatergic neurons expressing the Melanocortin receptor 4 (MC4R), while the neurons induced by reunion were primarily inhibitory neurons expressing the thyrotropin-releasing hormone receptor

(Trhr) (Figure 5). The activation of MPN neurons induced by isolation in mice housed in groups with satisfied social needs led to a significant increase in social interaction. Place preference assays showed that mice actively avoided the chamber associated with optogenetic activation, indicating that the activity of isolation-induced MPN neurons conveys a negative valence, potentially encoding the aversive emotional state linked to social isolation. The neurons projected mainly in the hypothalamus, including the PVN and VMH, as well as to other regions associated with aversive emotions, along with the motor relay PAG.[43] An important input to isolation-induced MPN neurons was identified from reunion-induced MPN neurons, suggesting their inhibition following social reunions.

A typical way how rodents engage in affiliative behavior is through social grooming or allogrooming, which involves touching each other with their forelimbs. This behavior is directed toward another individual and plays a vital role in building and strengthening social bonds in a wide range of social species, including primates. Rodent experimental evidence suggests that although subjects occasionally engage in allogrooming with unstressed partners, this behavior significantly increases when interacting with distressed partners.[73] It is important to note that self-directed grooming does not exhibit a corresponding increase, which argues against a non-specific rise in generic grooming behavior. Additionally, stressed partners exhibit a decreased social approach toward subjects compared to controls, indicating that the heightened allogrooming by subjects is not prompted by the stressed partners. When stressed partners are reunited with their counterparts, their anxiety levels decrease significantly, especially in the presence of allogrooming. This suggests a potent consoling effect, placing allogrooming among the prosocial behaviors in rodents. To address the mechanism by which this behavior is carried out, tachykinin-expressing GABAergic neurons of the MeA were selectively stimulated by optogenetics, which enhanced allogrooming. The study demonstrated the role of the MPOA in controlling elevated allogrooming behavior. This was achieved by selectively stimulating the preoptic terminals of tachykinin-expressing GABAergic neurons.[73] Another study found that social grooming was increased by a projection to the MPOA from parathyroid hormone 2-containing neurons of the posterior thalamus. Inhibition of the preoptic projection with chemogenetics was also effective suggesting the physiological role of the thalamo-preoptic pathway in promoting allogrooming behavior.[26]

In addition to homeostatic regulations, the reward system may also be involved in the control of affiliative behavior between conspecific of social species as social interactions are rewarding regardless of the sex of the other conspecifics. In an operant conditioning task where subjects nose-poked to gain access to and interact with a target animal, mice developed a strong preference for the social port. Both males and females showed a strong preference, suggesting that social interactions are intrinsically rewarding for both genders. Medial amygdaloid GABAergic neurons and their axonal projections to the MPOA displayed strong responses during social interaction. Furthermore, optogenetic studies have demonstrated that GABAergic neurons projecting from the MeA to the POA are necessary and sufficient for social reward.[20] The preoptic neurons that are activated by reunion have been identified as candidates for processing social interactions, linking homeostatic and reward processes. Optical stimulation of reunion-induced MPN neurons resulted in a place preference, indicating a positive association with their activities. Reunion-induced MPN neurons exhibited dense projections to the VTA and induced a significant release of dopamine in the nucleus accumbens during social interactions.[43] The neurons that are positive for neurotensin and project from the MPOA to the VTA are also potential conveyors of the presence of conspecifics as a reward, not just opposite-sex individuals as previously discussed. These neurons, which are predominantly GABAergic and ERα-positive, were found to be activated by both male and female urine odor. Non-social odors, including attractive food odors, had no effect. Moreover, optogenetic stimulation of preoptic neurotensin neurons elicits a rewarding response in the animal, as evidenced by heightened dopamine release, and promotes social behavior. This indicates that social approach modulated by neurotensin cells is rewarding for the animal.[41]

Aggression

Aggressive behavior in rodents, such as attacking a conspecific, can include aggressive mounting, pushing, and biting. These behaviors are most often experimentally elicited in a resident intruder test. As male rodents are generally more aggressive than females, most studies are carried out using males. If the males are not aggressive enough, wild or otherwise aggressive strains can be used, or aggression can be promoted by preceding social isolation of the animals.[74,75] It is also important to note that mothers demonstrated aggression toward an intruder. We will discuss this further in the Parental behavior chapter.

Contrary to the aforementioned behaviors, the MPOA is not the most crucial brain site for controlling aggressive behavior. Instead, male-male aggression is primarily controlled by neurons in the ventrolateral subdivision of the VMH (VMHvl) that contain ERα as well as progesterone receptors.[76,77] The MPOA is considered to be a part of the social behavior network[78] but not considered to be a component of the core aggression circuit.[79] Accordingly, lesions in the POA did not have clear effects on aggressive behaviors.[80,81] It is well-established that c-Fos activation occurs in the MPOA, particularly in its caudal part (cMPOA), after instances of aggression, as opposed to the more rostrally located neurons that are activated following sexual behavior.[76] Additionally, the density of neurons activated by aggression is similar to that of those activated by sexual behavior.[74] Based on single-cell sequencing, the neurons exhibiting aggression-induced c-Fos activation belong to two major clusters, both of which are GABAergic inhibitory neurons expressing ERα.[11] Limited information is available regarding the function of these neurons. *In vivo* calcium imaging of single cells demonstrated that the number of neurons in male mice responding to male conspecifics in the MPOA is relatively high, albeit less than those responding to females. Moreover, male respondents maintained this property during subsequent unrestrained social interactions.[67] Additionally, the neurons that are active during male mounting are distinct from those activated during female mounting.[37] The neurons that respond to males may potentially be involved in aggressive behavior toward them. Optogenetic stimulation of ERα-positive GABAergic MPOA neurons in male mice converted male-directed attack to mounting with ultrasonic vocalizations characteristic of female mounting. Indeed, long-range MPOA projections reach the VMHvl,[82] specifically, ERα-positive GABAergic MPOA neurons project to the VMHvl (Figure 5), and optogenetic stimulation of their terminals in VMHvl strongly inhibited

aggression.[37] Chemogenetic inhibition of ERα-positive cMPOA neurons resulted in repeated attacks toward female intruders, while their chemogenetic stimulation was consistent with optogenetic data as it nearly abolished intermale aggression.[44] Additionally, using a narrow optic fiber for fiber photometry to separately measure rMPOA and cMPOA activity revealed that rMPOA neurons responded to females, while cMPOA neurons responded to males. The latter response also depended on which male was used. The investigated animal elicited a larger response when previously defeated by a male.[44] These data suggest that MPOA neurons play a role in inhibiting aggression depending on the perceived strength of the opponent. To determine whether MPOA neurons transmit this information to the VMHvl to suppress aggression, monosynaptic rabies retrograde tracing from ERα-expressing neurons in the VMHvl was used to label cells in the cMPOA, indicating synaptic innervation of ERα-expressing neurons in the VMHvl by cMPOA neurons. Inhibition of the pathway evoked attacks even against a superior conspecific, confirming that the projection is necessary and sufficient for the control of attacks driven by VMHvl. Activation of the cMPOA to VMHvl pathway similarly suppressed attack in naive male mice from a naturally aggressive strain suggesting that the wiring of this pathway does not require adult fighting experience,[44] and can be considered an innate property.

HORMONAL INFLUENCE OF SOCIAL BEHAVIORS IN THE PREOPTIC AREA

The MPOA contains receptors for hormones and neuromodulators that are necessary for controlling instinctive behaviors. The MPOA is one of the major sites of action for several hormones such as estrogen, progesterone, prolactin, testosterone, as well as oxytocin, which acts in the MPOA as a neuromodulator. The distributions of receptors for these hormones vary within the MPOA, suggesting that the different hormones may affect different neuronal populations (Figure 4). It is worth noting that multiple cell types contain several of these hormone receptors,[11] indicating that different hormones may affect the same function and that each hormone may have an impact on various functions. Both excitatory and inhibitory neurons express receptors for various hormones and neuromodulators.[11] The expression level of hormone receptors in the MPOA exhibits sexual dimorphism in some cases,[83,84] and may also depend on the animal's state.[11] Indeed, an increase in ERα levels has been observed in the MPOA as a result of pregnancy[85] or reproductive experience.[86] Moreover, it was observed that a reduction in sexual behavior during the refractory period was linked to a decrease in the level of androgen receptor (AR) in the MPOA, which was subsequently restored upon recovery of sexual activity.[87] In addition, reinstatement of serum testosterone in aging males led to an increase in AR levels in the MPOA.[88] In turn, AR levels were also suppressed in the MPOA of mothers.[89] In contrast, it has been observed that during parturition[90,91] and during lactation[92] there is an increase in OTR levels in mothers' MPOA. Furthermore, hormones can affect each other's receptor levels, as evidenced by estrogen-induced progesterone receptor (PR) expression,[93] and progesterone's ability to elevate prolactin receptor levels (PRLRs).[94]

The alterations in hormone receptor levels may be relatively limited in scope if they do occur at all in relation to social behaviors. For example, studies have shown that sexual experience did not alter AR or ERα expression in the MPOA[96] and that no changes in either ERα or ERβ levels were detected in MPOA during pregnancy.[97] Furthermore, it has been found that maternal aggression did not alter PRLR.[98] The preoptic function is primarily regulated by hormones, which exhibit a wide range of changes depending on the animal's state, unlike their receptors. Maternal motivation, for instance, is induced by particularly high levels of estrogen and progesterone toward the end of pregnancy, with elevated prolactin and oxytocin also playing a role around parturition.[99] During pregnancy, hormones can alter the circuits responsible for parenting in preparation for future behavioral needs. For instance, estradiol can suppress MPOA galanin neurons, which are essential for maternal behaviors.[28] Additionally, progesterone has been found to permanently modify these neurons by promoting dendritic spine formation and the recruitment of excitatory synapses.[100] In turn, sexual steroid hormones do not play a significant role in maintaining maternal motivation after birth due to their low levels because of lactational anestrous.[101] Instead, it has been observed that prolactin and oxytocin are induced at parturition and subsequently by suckling,[102,103] which may affect maternal behavior in the postpartum period. Prolactin signaling is significant in the MPOA.[104] Suckling-induced prolactin signaling in MPOA neurons only partially overlaps with c-Fos activated neurons in the region.[105] Acute deletion of PRLR in all MPOA neurons of adult female mice resulted in profound deficits in maternal care soon after birth,[106] suggesting the importance and preoptic action of prolactin in forming maternal behaviors. The role of PRLRs in both maternal and paternal behaviors is crucial. Deletion of PRLR from CaMKIIα-expressing preoptic neurons resulted in a decrease in pup-retrieval behavior in fathers. Pharmacological inhibition of prolactin secretion was found to affect the ability to retrieve pups. However, the administration of exogenous prolactin was able to rescue this behavior in sires, suggesting that prolactin plays a crucial role in paternal behavior during interaction with pups.[107] Indeed, distinct oscillation patterns of tuberoinfundibular dopaminergic neurons lead to low dopamine release and consequently high serum prolactin levels in caring male mice.[108]

In contrast to prolactin, oxytocin is not able to cross the blood-brain barrier. Therefore, this neuromodulator can affect maternal behavior through oxytocinergic projections from the PVN that interact with OTR in the MPOA. However, under stress-free conditions, mice lacking OXT or OTR in the MPOA exhibited pup-directed behaviors that were similar to those of wild-type littermates. On the other hand, after restraint stress, they showed decreased pup retrieval, which suggests a possible role of the preoptic oxytocin system in maternal stress-coping behavior.[109] Preoptic OTR may also play a crucial role in lactation through PRL. The deletion of OTR in MPOA neurons prevented the elevation of PRL levels in plasma, which ultimately reduced the survival rate of the pups.[110]

Testosterone is recognized as a crucial factor in male sexual behavior and the promotion of aggressive behavior. It acts on preoptic AR, as well as on ERα following local aromatization to estrogen. To determine the site of these actions, ERα expression was locally downregulated using an adeno-associated virus vector expressing a small hairpin RNA targeting ERα. Suppressing ERα in the MPOA resulted in a reduction of sexual behavior while leaving aggressive behavior unaffected. On the other hand, in the VMN, it reduced both behaviors. However, the knockdown of ERα in the MeA did not have any effect on either behavior.[81] Additionally, testosterone plays a crucial role in the sexual differentiation of rodents' brains during the perinatal period, including the formation of sexually dimorphic brain regions within the MPOA.[111]

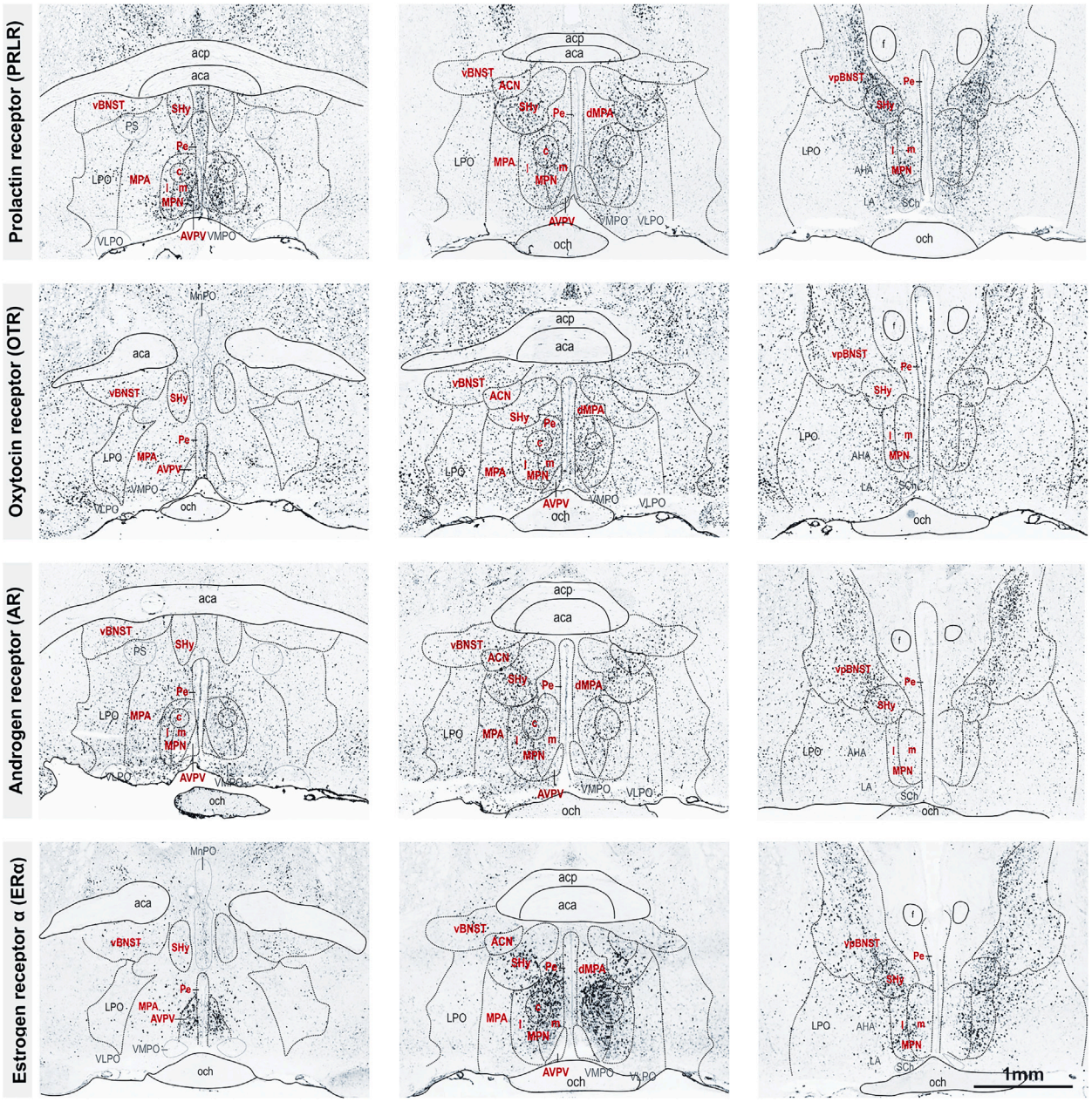

Figure 4. The distribution of major socially relevant hormone receptors in the preoptic area (POA) in the mouse brain

The figure shows the distribution of prolactin receptor (PRLR), oxytocin receptor (OTR), androgen receptor (AR), and estrogen receptor α (ERα) mRNA obtained by *in situ* hybridization, as reported by the Allen Mouse Brain Atlas.[95] The selected sections display the expression of each hormone receptor in sexually naive mice, at three different antero-posterior levels located between bregma levels +0.3 and −0.3 mm. PRLR and ERα are particularly abundant in the AVPV and the MPOA. AR has a distribution similar to ERα with lower expression levels in the MPN, while OTR distribution is relatively less pronounced but more widespread in the POA.

NEURONAL INPUTS TO THE PREOPTIC AREA AFFECTING SOCIAL BEHAVIORS

Social interactions in the MPOA are governed by input from conspecifics, which is primarily transmitted through olfaction. The MOA receives neural projections from the olfactory and accessory olfactory bulbs, which detect social olfactory cues through the main olfactory and vomeronasal systems, respectively.[112] Instead of direct connections, the MPOA receives olfactory inputs via the MeA, in which largely separate neuronal populations are activated by males, females, and pups.[113] A GABAergic cell population in the MeA projects to the MPOA, mediating social reinforcement behavior and dopamine release in the nucleus accumbens.[20] In addition to the MeA, the principal subdivision of the bed nucleus of the stria terminalis (BNSTpr) also conveys olfactory input to the MPOA, which the BNSTpr receives directly from the main

olfactory and vomeronasal systems as well as from the MeA. Distinct populations of female-versus male-tuned neurons are present in the BNSTpr.[67] A subpopulation of the aromatase-expressing BNSTpr neurons, those expressing tachykinin 1 (Tac1) were found necessary for sex identification of a conspecific.[40] In male mice, aromatase-positive BNSTpr neurons were required for mounting and attack.[114] Furthermore, optogenetic inhibition of the projections from Tac1-expressing BNSTpr neurons to the MPOA suppressed mating behavior, indicating the significance of these MPOA inputs in male sexual behavior.[38] In addition to sexual behaviors, olfactory inputs also promote allogrooming, a major component of direct contact affiliative social interactions. Olfactory cues from a stressed donor mouse were transferred to a naive partner via an anogenital swab, which led to increased allogrooming by the subjects toward their partners. This information was relayed to the MPOA via a tachykinin-expressing subpopulation of GABAergic neurons in the MeA.[73] Finally, olfactory inputs play an important role in maternal behaviors, especially after giving birth, as signals from the offspring become crucial for maintaining maternal motivation. Therefore, neuronal inputs from pup cues take over the role of hormones in maintaining maternal motivation in the postpartum period.[115]

The significance of somatosensory input was shown by the effects of its elimination. Isolation of females from free social interactions but maintaining interactions via other modalities induced active contact-seeking behavior while reunion with peers induced physical contact.[42] The elevated physical contact, also called social rebound was inhibited by intraperitoneal injection of isoguvacine just before reunion.[43] Isoguvacine is a peripherally restricted $GABA_A$ receptor agonist that attenuates peripheral mechanosensory neuron firing. Therefore, its effectiveness in eliminating social rebound also suggests the importance of somatosensory inputs in peers-induced physical contact behavior.[43] Comparing the behavioral responses to acute social isolation and reunion between sexes, it was found that male mice exhibited significantly less contact-seeking upon social isolation. Upon reunion, male mice contacted each other to a similar extent as females, but their interactions were more aggressive and less affiliative compared with females,[116] in which social grooming dominated as a highly affiliative direct contact behavior.[26] The preoptic processing of elevated social contact following reunion is evidenced by the increased number of c-Fos-activated neurons in the area.[26,116] Further evidence for the social activation of these cells was provided by *in vivo* calcium imaging, which showed a population of isolation and another population of reunion-activated neurons in the MPN.[43] To further investigate the specific role of somatosensory input, mice were exposed to plastic tunnels internally coated with soft cloth material. Interestingly, the activity of isolation-induced neurons was inhibited while reunion-induced neurons were excited during crossing the tunnels.[43] These results indicate that gentle touch is effective in reducing isolation- and increasing reunion-induced neuronal activity.[117] A candidate for how these cells affect upper brain centers was suggested to occur via the external lateral subnucleus of the lateral parabrachial nucleus (PBL). PBL neurons were indeed activated by social touch, however, ablation of Mrgprb4 receptor neurons showed only a slight reduction in social rebound compared to wildtype controls. In addition to the PBL, the posterior intralaminar thalamic nucleus (PIL) emerged as a candidate to convey social information to the MPOA. This region receives input from the spinal cord and is activated by social interaction involving direct physical contact.[30,103] The major output of the PIL is the MPOA. Furthermore, experimental activation of preoptic projecting and social activity-tagged neurons in the PIL increased while their inhibition decreased social grooming.[26] Somatosensory input also plays a role in the activation of preoptic neurons in the mother following pup exposure. The mothers receive intense somatosensory input in the form of suckling. If direct contact with the pups is prevented, a lower number of preoptic neurons are activated[51,118] suggesting the contribution of suckling to pup-exposure-induced activation of preoptic neurons. While the anatomical pathway involved in this activation is not fully established, the PIL is a candidate to convey suckling input to the MPOA. PIL neurons are activated by suckling in the mother with reduced activation in the absence of direct contact with the pups.[119,120] In turn, a neuropeptide, tuberoinfundibular peptide of 39 residues, also called parathyroid hormone 2 is activated in PIL neurons, which project to the MPOA,[121] synapsing on galanin[29] and oxytocin neurons.[30] While the receptor of this neuropeptide, the parathormone 2 receptor is abundant in the MPOA,[122] its local blockade with virally expressed antagonist reduced maternal motivation.[120]

In addition to the inputs to the MPOA described above, anatomical studies determined several other significant afferent connections of the MPOA.[123,124] These results have been largely confirmed by more recent cell-specific connectional studies using monosynaptic retrograde viral tract tracing. Preoptic glutamatergic neurons received the strongest inputs from the lateral septum, the BNST, and the PVN.[18,27] Galanin neurons also received strong input from these brain regions, but even more inputs terminate on galanin neurons from the arcuate nucleus and the VMH.[31] Furthermore, galanin neurons receive several additional significant inputs. Since different galanin populations exist,[11] it is not surprising that their inputs are also different,[31] which probably models other types of preoptic neurons suggesting highly complex connectivity. Research groups have only recently begun to investigate the functional inputs to the MPOA.[35,39] For instance, the MPOA input from the amygdalohippocampal transitional area was shown to inhibit caring.

FUTURE DIRECTIONS IN UNDERSTANDING PREOPTIC MECHANISMS OF SOCIAL BEHAVIORAL CONTROL

The instinctive social behaviors controlled by the MPOA are hard-wired, therefore relatively simple but effective. Different neuronal subcircuits have evolved, which are responsible for carrying out reproductive, affiliative, and aggressive social behaviors (Figure 5). As previously discussed, our understanding of these regulatory mechanisms has advanced considerably in recent years. However, despite the identification of a considerable number of cell types within the POA, only a limited number of circuits have been subjected to investigation[11] suggesting that more discoveries are needed to fully understand the control of instinctive behaviors. For example, each behavior is comprised of distinct components (e.g., aggression is comprised of biting vs. lounging toward an intruder), which may be partially independently regulated. Automated behavioral analyses may enable researchers to reproducibly detect subtle changes in behavior and connect them to neuronal functioning.[125,126] Furthermore, cell groups have only been studied based on their common genetics, with the focus being on common promoters. It is therefore anticipated that functional assemblies of neurons will form for fine-tuned control of behavior, as demonstrated in other brain regions.[127] It is similarly anticipated that the neuronal circuitry regulating different behaviors will interact with each other. For

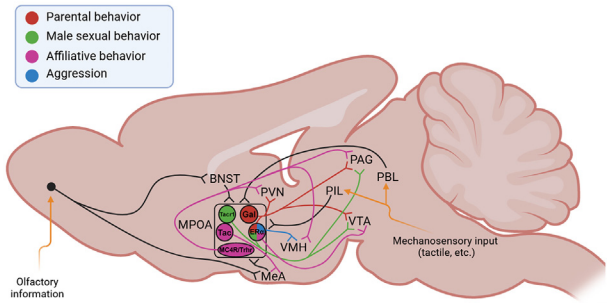

Figure 5. Summary of functionally characterized cell types and connections of the medial preoptic area (MPOA) involved in the regulation of social behavior

Galanin (Gal)-positive neurons and their projections regulating parental behavior are marked in red. Tachykinin receptor 1 (Tacr1)-expressing neurons and their projections regulating male sexual behavior are shown in green. Several cell populations have been identified that are responsible for the regulation of affiliative behavior, such as melanocortin receptor 4 (MC4R)-, thyrotropin-releasing hormone receptor (Trhr)-, and tachykinin (Tac)-expressing neurons. These cell populations and their projections are shown in purple. While these cell groups likely represent non-overlapping neuronal populations, estrogen receptor α (ERα) is expressed in a large number of MPOA neurons with heterogeneous functions. ERα-expressing neurons have been implicated in all types of social behavior. In cases where a particular projection of ERα neurons has been shown to be involved in a particular type of social behavior, the projections are indicated by the color corresponding to that social behavior. In addition, the blue color indicated the projection from ERα neurons to the ventromedial hypothalamic nucleus (VMH) regulating aggression. Note that the indicated cell groups have projections to several other brain regions that are not indicated due to lack of available functional information. Similarly, additional types of input other than olfactory and mechanosensory are likely but are not indicated in the summary figure due to lack of available knowledge about them. Other abbreviations: BNST: bed nucleus of the stria terminalis, MeA: medial amygdaloid nucleus, PAG: periaqueductal gray, PBL: parabrachial nucleus, PIL: posterior intralaminar thalamic nucleus, PVN: paraventricular hypothalamic nucleus, VTA: ventral tegmental area.

instance, an animal cannot exhibit both affiliative and aggressive behaviors simultaneously. The mechanisms underlying these interactions between the responsible neuronal circuitries are expected to emerge in future studies. Finally, social behavior is not solely controlled by instinctive preoptic mechanisms but also by cognitive processes controlled by the forebrain, particularly cortical brain regions. Although the significance of these cortical regulations is less pronounced in rodents than in humans, rodents do possess cortical social behavioral control elements, including social recognition, social memory, internal state recognition, and even empathy-like behavior.[128] The interconnection of forebrain and preoptic regulation has not been thoroughly studied, yet it must be addressed in future animal studies. For example, the medial prefrontal cortex is known to project to the MPOA[129] but the functions of these projections remain elusive. Additionally, in humans, although cortical processes primarily determine complex social interactions, social interactions involve cognitive components such as social memory and, for example, mentalization, advanced communication, and social decision-making,[130] preoptic mechanisms are expected to exist and exert their influence on social behavior. It will be of interest to ascertain their function in future human studies. Finally, the motor output of the instinctive behaviors has to be investigated in more detail. It does not seem to involve the motor cortex but rather joins the medial or emotional motor system, whose major component is the PAG.[131,132] Moreover, the POA can elicit behavioral responses through the reward system, specifically via the VTA to nucleus accumbens pathway[22] (Figure 5). However, the execution of the different behavioral responses remains poorly understood, necessitating further investigation in future studies. *In conclusion*, the MPOA is emerging as one of the major brain centers of instinctive social behaviors. Indeed, its major amygdalar, thalamic, and brainstem inputs avoid conscious processing in the cerebral cortex to affect social contact. While key elements of MPOA circuits have been identified, a synthesis of these new data is now provided. In addition, future studies are proposed to reveal the mechanisms of how the area controls social interactions.

LIMITATIONS OF THE STUDY

Female sexual behavior is largely controlled by the ventromedial hypothalamic nucleus. However, important aspects are influenced by preoptic neurons that were not discussed in the article. Another limitation is that we omitted theoretical considerations on the preoptic cell type used for opto- and chemogenetics and those identified by single cell and spatial sequencing. Finally, potential human homologue mechanisms were not discussed.

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AUTHOR CONTRIBUTIONS

T.L.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review and Editing, Visualization. **D.D.**: Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review and Editing, Visualization. **S.O. and G.P.**: Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review and Editing, Visualization, Funding acquisition. **A.D.**: Conceptualization, Methodology, Resources, Writing - Original Draft, Writing - Review and Editing, Supervision, Project administration, Funding acquisition.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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