Simultaneous control of pup-directed and depression-like behavior by preoptic inhibitory neurons in mice

Ph.D. thesis

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Introduction

Parental caregiving serves as an essential set of behaviors towards an immature conspecific, increasing the likelihood that the developing offspring will survive to maturity. The diverse range of parental strategies is predominantly influenced by the type of mating system that a species displays. Most mammals including rodents exhibit a polygynous mating system, typically associated with a female-dominated uniparental care strategy. While in monogamous mammalian species like humans, both mothers and fathers are equally part of offspring care, in such non-monogamous mammalian species, males are separated from females after mating and not involved in raising the litter. From an evolutionary perspective, the driving force behind the mating program displayed by the male members of polygynous species is to increase their reproductive success by mating with several partners as well as by showing aggression even infanticide towards alien pups. Under natural conditions, when exposed to pups, virgin females react to them as an aversive stimulus and try to avoid or attack them, similar to the behavior exhibited by male animals. Despite the high prevalence of infanticide in feral females, laboratory nulliparous female mice show caring behavior as much as mother animals do, nearly immediately,

probably due to inbreeding. Since virgin females do not experience pregnancy associated with hormonal changes to trigger maternal responsiveness, there must be alternative routes through which generic infant stimuli gain access to the brain circuits that control parental attraction. This fact would have been the case during human evolution and allowed the possibility of adoption. The emergence of alloparental behavior in both species provides further evidence that the neuronal network underpins the main aspects of maternal motivation is evolutionary conserved and likely to be quite similar in all mammals, making laboratory animals a good candidate to study the neuronal basis of parental behavior.

Among the brain regions controlling the sensation and motoric reactions to infants, the rostrally located hypothalamic medial preoptic area (MPOA) is known as a crucial node for the expression of maternal behavior in rodents and humans as well. Although most of the studies highlighted the prevalent role of the MPOA in the caregiving behavior have been performed on laboratory mice and rats, an increasing number of literature from primate and human research supports the importance of this brain region in parenting too. In rats, depression of the MPOA during the postpartum period significantly reduced the maternal motivation of mother animals indicated by the disruption of retrieval behavior,

nursing behavior and nest building. Furthermore, inactivation of the neuronal efferent of the MPOA during pregnancy prevents the onset of maternal behavior at parturition suggesting that the activity of the MPOA is essential for both the onset and the maintenance of maternal behavior in laboratory animals. However, when something is to go wrong with the physiological activity of the parenting circuit, there are devastating consequences for the welfare of both mother and her child. In human society, 6.5%-20% of women around the world experience the severe symptoms of postpartum depression, most of the mothers are left alone without proper help and medications. This high prevalence of depression in the postpartum period indicates that maternal alterations in the brain necessary for taking care of the offspring somehow increase the chance of the development of depression. As a converging centrum, the study of the MPOA may help to understand how maternal circuits became dysfunctional and develop a targeted therapy.

Importantly, the MPOA is a heterogenous brain region with a high expression of numerous receptors for known modulators of parenting and with a diverse projection map. Due to the diversity of the MPOA, the use of correlational methods and cell type-specific manipulations provide a huge advantage to understand how parenting is organized at the

neural level and how the potential dysregulations impact the caregiving behavior. An important correlational histology-based method that has been used for a long time to make a connection between behavior and neuronal responses is the detection of the protein product of the immediate early gene *c-fos*. The detection of the c-Fos allows us to precisely locate neurons that reacted to a certain stimulus, like exposure to pups. Numerous studies have shown that the expression of c-Fos significantly increases in the MPOA of mother rodents following pup exposure suggesting that this brain region is activated due to maternal behavior. More precisely, Lonstein and De Vries have reported that more than half of the whole preoptic neuronal population expresses the synthesizing enzyme for the inhibitory neurotransmitter γ-aminobutyric acid (GABA). The important role of the preoptic inhibitory circuit in the expression of parenting is also supported by tracer studies. In detail, the MPOA sends projection to the brain regions regulating the defensive behavior towards pups, proposing that one of the certain functions of the preoptic neurons is to inhibit brain regions promoting infanticide. Although the pup-induced increased activity of preoptic inhibitory neurons supports the possibility of the abovementioned hypothetical role of the preoptic inhibitory

neurons, for a long time we do not have direct neurobehavioral evidence of it.

Objectives

Taking into consideration the gap in knowledge about the mechanisms of parental care and the connection between motherhood and mood disorders, we set four specific goals to reveal a previously unknown player in the development of depression by expanding our interest in the potential involvement of preoptic inhibitory neurons.

Our first aim was to map the distribution of preoptic GABAergic neurons activated by pup exposure in both female and male mice with different reproductive states to explore whether the pup-induced activity of preoptic inhibitory neurons shows sexual dimorphism or internal state-dependent pattern.

Secondly, since the preoptic GABAergic neurons are highly activated following pup exposure, we hypothesized that the chemogenetically induced activity changes of these cells are manifested in behavior outputs. To test our hypothesis, we performed different behavior tests measuring behavior responses towards pups following the cell type-specific activation or inhibition of preoptic GABAergic neurons in both sexes as well as in sexually inexperienced animals and parents.

The next question aimed to reveal the presynaptic partner of the preoptic GABAergic neurons to understand how the activity of these neurons is regulated under normal conditions. Furthermore, by mapping the projections of preoptic GABAergic neurons we sought to anatomically support the role of these cells in connection to parental behavior.

Last, but not least, considering the high prevalence of mood disorders during the peri- and postpartum periods, we aimed to examine the activity changes of preoptic GABAergic neurons following stress exposure and the potential involvement of the preoptic inhibitory circuit in both anxiety and depression-like behaviors in connection to parenting by using correlative studies.

Methods

To investigate the effect of pup exposure and stress on neuronal activity, we took advantage of the stimulus-evoked rapid expression of c-Fos protein and analyzed the percentage of c-Fos-expressing GABAergic cells visualized by fluorescent immunolabeling against the c-Fos in genetically modified mice expressing ZsGreen fluorescent protein driven by the vesicular GABA transporter (VGAT) promoter.

Using designer receptors exclusively activated by designer drugs (DREADD)-based technology allowed the cell-type

specific manipulation of preoptic VGAT-positive neurons. This way, we had the opportunity to explore the effect of bidirectional changes in the neuronal activity of preoptic GABAergic cells on pup-directed behaviors, anxiety-like behaviors, and depression-like behaviors simultaneously, in both sexes with different internal states.

Both the hormonal and non-hormonal factors affecting the activity of preoptic inhibitory cells were examined using a variety of techniques to provide not only anatomical but physiological evidence for the integratory role of this neuronal population, including immunohistochemistry, pre-and post-embedding immunogold labeling for electron microscopical investigation and *in vitro* electrophysiology recordings.

The brain-wide connections of the preoptic GABAergic neurons were revealed by cell-type specific anterogradely spreading AAV injection, and a retrogradely spreading AAV injection into the MPOA administered via intracranial surgeries.

Results

In the present study, we observed different neuronal responses of preoptic inhibitory neurons to pup exposure in relation to sex and reproductive stage using c-Fos protein as a marker for neuronal activation. Significantly more GABAergic

neurons expressed the protein product of the immediate early gene *c-fos* in virgin females, mothers, virgin males, and fathers than in the sex-matched control groups, who had no interaction with pups. Notably, both sexually inexperienced males and father animals showed increased neuronal activity, the magnitude of the pup-induced c-Fos expression was significantly weaker than in female mice. Furthermore, by dissecting the MPOA to spatially distinct subpopulations, we revealed uneven neuronal activity patterns among the subregions of the MPOA, with the highest number of c-Fos expressing VGAT-positive neurons in the medial part of the mother animals, but not in fathers.

In addition, we established the sexual dimorphic role of the preoptic GABAergic neurons in the regulation of both parenting and anxiety-like behavior. In nulliparous female and mother mice, the activation of the preoptic inhibitory neurons promoted intensified maternal behavior and parenting-linked anxiety, contrary, in male animals, the same approach induced pup-and also male-directed aggression without affecting the level of anxiety measured by two different behavior assays. The other way around, inhibition of the preoptic inhibitory neurons significantly reduced the different aspects of caregiving behavior in female animals leaving unchanged the

anxiety-like phenotype, while increasing the parental interactions in father animals, but not in virgin males.

In contrast to the sexually different role of preoptic GABAergic neurons in parenting and anxiety, the selective stimulation of these neurons exerts the same effect on both females and males in promoting depression-like behavior indicated by the increased duration of immobility during the forced swim test. To further support the involvement of the preoptic inhibitory pathway in the regulation of the depression state, we also investigate hedonic behavior in female mice. In good agreement with our previous findings, the excitation of preoptic GABAergic cells resulted in decreased sucrose consumption, which is an indicator of anhedonic behavior.

Finally, we also provided both anatomical and physiological evidence supporting the role of preoptic inhibitory neurons in parenting and depression throughout their potential inputs and outputs. In more detail, preoptic GABAergic cells have a bi-directional connection with brain regions involved in the regulation of parenting and emotional states. Among all the brain regions, the oxytocinergic neurons-expressing paraventricular nucleus (PVN) receives the most robust projections from the preoptic inhibitory cells. Interestingly, despite the high density of preoptic GABAergic fibers in the PVN, following retrogradely-spreading AAV

injection into the MPOA, significantly more retrogradely labeled cells are found in the supraoptic nucleus, another brain region with a high abundance of oxytocin-positive cells. *In vitro,* patch clamp measurements confirm that preoptic VGAT-positive neurons react to oxytocin with a higher firing frequency. In addition to the inputs derived from oxytocinergic cells, tuberoinfundibular peptide 39 (Tip39)-containing fibers also make synaptic connections with the preoptic inhibitory neurons. Moreover, double immunolabeling revealed that half of the preoptic GABAergic population expresses the nuclear transcription factor estrogen receptor alpha, which can be activated by the sex hormone estrogen.

Conclusions

Although postpartum neuropsychiatric disorders are a major source of child abuse, none of the previous neurobiological investigations has focused on the simultaneous examination of parenting and depression, especially in the mirror of sex differences.

Investigation of the activity pattern in both sexes implies that the distinct behavior repertoire displayed by males and females towards pups is coded by the different hypothalamic engrams. The activity of these neurons is potentially regulated by both hormonal and non-hormonal influences making the

preoptic GABAergic neurons a good candidate for the integration of information coming from the outer and inner world. Moreover, exploring the projections of the preoptic GABAergic neurons assumed that this neuronal population may exert its effect through brain regions that are involved in the control of pup-directed or emotional behavior. Among all the brain-wide connections of preoptic inhibitory neurons, their reciprocal relationship with the oxytocinergic system may be the link between the simultaneous regulation of parenting and depression behaviors. Further, we revealed the opposite action of preoptic inhibitory neurons in parenting and parenting-linked anxiety-like behavior, also, confirming their sex-independent participation in depression. The increased level of depression following the chemogenetic activation of pup-responsive preoptic inhibitory neurons proposes that the impairment of the preoptic GABAergic circuit would cause disinterest instead of caretaking behavior in association with the development of depression.

In conclusion, our data suggest that the preoptic inhibitory network underpins the well-known sex differences in parenting behaviors of polygamous mouse species including the parenting-linked anxiety-like phenotype observed only in female animals. Also, the involvement of the preoptic GABAergic neurons in the control of different aspects of

depression-like behavior supports the view that the rewiring of maternal circuits during pregnancy and the postpartum period makes mothers more vulnerable to depression as the neuronal networks regulating the caregiving and mental health are shared.

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