Chapter 1

Introduction

Genetic control of Innate behaviors

Ethologists postulate that all animals possess the abilities to ingest, reproduce, and defend against predators and threatening stimuli in order to survive as individuals and also as a species. These three forms of behavior are called innate behaviors, and they are characterized by several distinct features.

First, it is assumed that animals are born with these innate behaviors, thus requiring no learning. Second, they are released by a specific stimulus and result in a fixed action pattern. Third, these behaviors are so crucial for the survival of animals, that selection has generated a genetic mechanism to specify these behaviors.

Innate behaviors can be more or less complex. This is well illustrated by the reproductive instinct in the tree-spined stickleback fish described by the Nobel Prize-winning ethologist, Nikolaas Tinbergen (Tinbergen, 1951). In males, reproductive behaviors are choreographed in such a way that each action is built into a hierarchical organization and follows a precise sequence of actions. Thus, if the sequence is interrupted at some point, none of the downstream events are expressed. Can such a complex repertoire of behaviors be specified by dedicated regulatory genes?

Genes can function to specify behaviors in two ways. First, they can orchestrate the actual manifestation of behaviors. Second, they can function to construct neural circuits, which in turn control behaviors (Baker et al., 2001). If we focus on the second meaning of genetic control of behaviors, it is suitable to state that innate behaviors are controlled by genes. If an animal is born with an ability to execute a fixed action pattern in response to a fixed range of stimuli, it is plausible to assume that the circuits controlling these behaviors are genetically hard-wired into the brain of the animal during its development.

Which part of circuits do genes specify then to control a particular behavior, if we consider 'input → neural processing → output' scheme? The sensory modalities that detect stimuli or receive input work more or less in a generic fashion. They are not utilized to elicit a specific type of behavior, but rather a broad range of behaviors. In the same manner, central pattern generators, which are neuronal ensembles capable of producing basic motor outputs (Arshavsky et al., 1997), are also largely generic. Thus, genes controlling behaviors are likely to operate on the part of the circuit, which integrates input, processes it, and modulates central pattern generators to coordinate proper behavioral output (Baker et al., 2001).

Then, how does one go about finding genes that specify dedicated circuits for controlling specific behaviors? One can use a top-town approach; one can screen for behavioral mutants and locate the areas in the brain where the mutant genes function. One then can infer the function of those areas and the circuits, in which the brain areas are embedded, from the mutant phenotypes. I took a different approach; I took two already known circuitries which are dedicated for the expression of rodent reproductive and defensive behaviors, and searched for the genes that are expressed in part or in all of the circuit components, expecting that some of them might function to specify the circuits themselves.

Reproductive and defensive behaviors

The terms 'reproductive behaviors' and 'defensive behaviors' are used broadly in this manuscript. Defensive behaviors include sets of behaviors for animals attacking a

conspecific and for animals under attack. They also encompass a second model of defense: antipredator behaviors (Blanchard and Blanchard, 1988).

Reproductive behaviors include masculine sexual, feminine sexual, and parental components. Since the qualitatively same amygdalar-hypothalamic circuitries controlling reproductive behaviors are present both in males and females, albeit the differences in the sizes of the nuclei involved and in the densities of the projections (Simerly, 2002), I did not pursue sexual dimorphism in the behaviors. Accordingly, I limited my study to males because their reproductive behaviors are not influenced by cyclic hormonal changes.

Male sexual behaviors are subdivided into mainly two phases: sexual arousal and performance (Beach, 1956; Beach, 1966; Beach, 1976; Swanson, 1987). The initial reaction of males to receptive females usually consists of thorough chemoinvestigation of the females. Crossing the copulatory threshold in this initial phase results in mounting, intromission, and eventual ejaculation (Beach, 1956). The chemoinvestigation is usually accompanied by courtship vocalization at ultrasonic ranges (68 kHz, sequences of 50- to 300-ms pulses) (GD, 1972; Whitney et al., 1974). The calls continue into the copulatory phase and finally cease after the male has ejaculated (GD, 1972).

Lastly, it should be noted that there are motivational aspects (drives, appetites, urges) to both reproductive and defensive behaviors that have not been covered in my study. I focused on the motor outputs of reproduction and defense rather than their affective aspects. The biological significance of amygdalar-hypothalamic circuits in regulating affective aspects of these behaviors are still unanswered.

Vomeronasal Organ (VNO)

Semiochemicals as releasers of innate behaviors

Olfactory stimuli play an important role in the release of reproductive and defensive behaviors. Semiochemicals are the molecules used for communication between animals, and they serve as such olfactory stimuli (Wyatt, 2003). Most vertebrates detect the semiochemicals using a dual olfactory system: the main olfactory epithelium, projecting to the main olfactory bulb in the brain, and the vomeronasal organ (VNO), projecting to the accessory olfactory bulb (AOB) (Itaya, 1987).

While recent genetic evidence increasingly suggests an important role for the main olfactory system in processing semiochemicals that elicit innate social behaviors (Belluscio et al., 1998; Keverne, 2002b; Leypold et al., 2002; Stowers et al., 2002), a great deal of attention has been focused on the parallel pathway involving the accessory olfactory system, which specifically responds to pheromonal cues (Brennan and Keverne, 2004; Luo and Katz, 2004; Newman, 1999). Pheromones are a subclass of semiochemicals that are secreted by an individual to communicate social and reproductive status within the species and release stereotyped behaviors or developmental processes from the receiving individual (Wyatt, 2003).

Surgically removing the VNO eliminates male courtship ultrasonic responses to females and reduces male sexual behaviors, male-male aggression, and maternal aggressive behaviors (Keverne, 2002a; Wysocki and Lepri, 1991). Genetic manipulations resulted in similar phenotypes, albeit to varying degrees. Deleting *TRP2* ion channels, thus eliminating VNO neuronal activity, completely abolished both male-male and maternal aggressions. In

addition, male mice deficient in *TRP2* expression initiated sexual and courtship behaviors toward both males and females, suggesting that the functional VNO is necessary for gender discrimination (Leypold et al., 2002; Stowers et al., 2002). Mice lacking a cluster of vomeronasal receptor genes also displayed deficits in the expression of male sexual behaviors and maternal aggression, although male aggressive behavior was normal (Del Punta et al., 2002a). Thus, the foregoing data is consistent with the interpretation that the VNO plays major roles in detecting olfactory stimuli generated by conspecifics and in subsequently eliciting appropriate reproductive and defensive behaviors.

Recent data suggested that VNO is not limited to detecting pheromones, semiochemicals used to communicate within the species by definition. Cat odor-exposed rats showed a substantial increase in the c-fos expression in the AOB and its main projection target, MEA (McGregor et al., 2004). These data suggest that cat odor is processed like a pheromone–like stimulus by the vomeronasal system of the brain. Consistent with this interpretation, cat odor failed to prevent play behavior in rats with the sectioning of the vomeronasal nerve, whereas it remained an effective anxiogenic stimulus when the damage was made to the main olfactory system (Panksepp, 1998). Combined with the data showing that the behavioral responses released by cat odor in rats are instinctive and stereotypical in nature, the foregoing observations suggest that the VNO might function to process chemical signals operating across species to elicit innate behaviors.

Pathways from VNO to MEA

As mentioned in the previous section, the olfactory stimuli perceived by the vomeronasal receptor neurons in the VNO are subsequently relayed to the AOB (Itaya, 1987),

which in turn projects heavily to the MEA (Davis et al., 1978; Kevetter and Winans, 1981; Krettek and Price, 1978; Scalia and Winans, 1975; (Luo and Katz, 2004) (Figure 1). Tracing studies indicate that the dendrites of the AOB mitral cells (second order projection neurons in the VNO pathway) innervate only the glomeruli receiving input from common vomeronasal receptor neurons, thus implying that each AOB mitral cell will be activated by only one receptor type (Del Punta et al., 2002b). The foregoing data combined with the fact that the vomeronasal receptor neurons show highly selective responses (Holy et al., 2000; Inamura et al., 1999) predict that the second order projection neurons will be activated by a small, specific set of semiochemicals as well. Keeping with this prediction, they showed a high degree of selectivity for the sex and strain of stimulus animals (Luo et al., 2003).

The pattern of immediate early gene activation showed that the mitral cells also respond to fundamentally different types of chemosensory information depending on their location within the AOB or the classes of vomeronasal receptor neurons they are innervated by. Anterior AOB is innervated by the VIR-expressing vomeronasal neurons, whereas the posterior AOB is innervated by the V2R-expresing neurons (Belluscio et al., 1999; Dulac and Axel, 1998; Rodriguez et al., 1999). Exposure of a male mouse to a female in diestrus results in the activation of cells in the anterior AOB (Dudley and Moss, 1999; Kumar et al., 1999). On the other hand, male-male interaction resulting in the aggressive behaviors selectively activated a population of neurons located in the posterior AOB (Kumar et al., 1999). Cat odor, considered to be a defensive stimulus to rats, also elicited mitral cell activation specifically in the posterior AOB (McGregor et al., 2004) These results suggest that the anterior AOB may be primarily activated by semiochemicals related to reproductive behaviors, while the posterior AOB processes cues relating to defensive behaviors. Thus, the

results suggest that the anatomically segregated pathways from the VNO to AOB are also functionally distinct based on the semiochemicals they respond to.

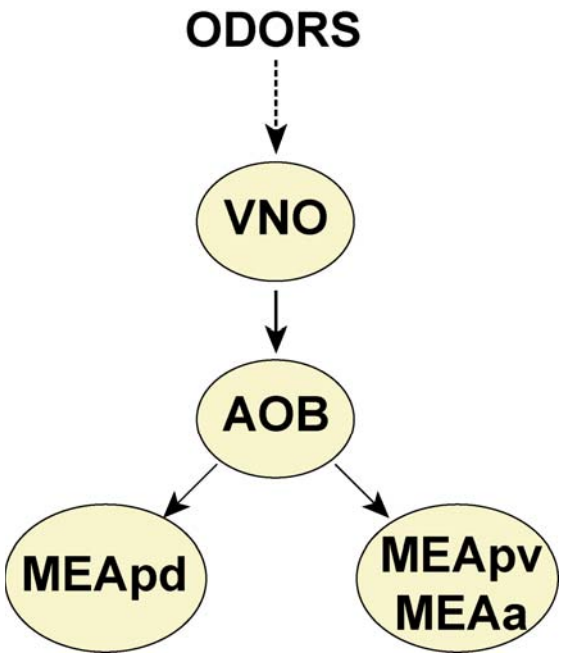

Figure 1. Projections of the VNO.

Vomeronasal receptor neurons of the VNO project to the accessory olfactory bulb (AOB), which in turn sends its axon to the MEA nuclei, including MEApd, MEApv, and MEAa.

Circuit Components

Medial Amygdala (MEA)

The Amygdala is an arbitrarily defined, heterogeneous group of nuclei located near the temporal lobe of the mammalian cerebral hemisphere. The nuclei are derived from both the cortex and the striatum, and they serve several functions, including accessory olfactory, main olfactory, and autonomic functions (Swanson and Petrovich, 1999). It has also been extensively studied for its role in modulating emotion, especially in the context of fear. Central amygdala and basomedial complex of amygdala are known to mediate conditioned fear responses in rodents. On the other hand, medial amygdala (MEA) is hypothesized to be part of the circuit orchestrating innate fear responses.

The MEA is comprised of several subnuclei: medial amygdala anterior, medial amygdala posterior dorsal (MEApd) and medial amygdala posterior ventral (MEApv). The MEA is unique among the amygdalar neurons in that it is one of the few projection targets of the AOB that transmits pheromonal and other chemosensory information for eliciting innate behaviors, as discussed above. Consistent with this observation, various functional studies support the essential role of the MEA in mediating the fearful or innate defensive responses.

Studies using an immediate early gene *c-fos* expression identified the MEA as one of the neural substrates brought into play when animals are placed under social stress, such as an encounter with an aggressive resident male (Chung et al., 1999; Chung et al., 2000). Other studies demonstrated that the MEA responded also to predatory cues. Experiments using predatory odor showed that olfactory stimulus (cat odor) was sufficient to elicit defensive behaviors from rats, which avoided the source of the predatory odor and spent a

greater amount of time in the hide box. In these animals, the *c-fos* expression was specifically induced in the MEApv, implicating its possible role in mediating defensive behaviors (Dielenberg et al., 2001; McGregor et al., 2004).

Supporting the role MEA plays in the expression of the innate defensive behaviors, MEA lesions abrogated predator odor-induced unconditioned fear responses in the rat. Rats with damage to the MEA exhibited reduced freezing responses after exposure to the cat odor, and also made frequent contact with the source of the odor. The effect was specific to the MEA as the damage to the central nucleus, which is implicated in mediating conditioned fear responses, failed to results in the same deficit in the innate defensive behaviors (Li et al., 2004).

Electrically stimulating the MEA demonstrated that it plays a facilitating role in producing defensive behaviors. Dual stimulation of the MEA significantly decreased the latencies for defensive behaviors elicited from the single stimulation of the medial hypothalamus (Shaikh et al., 1993). Thus *c-fos* expression studies and lesion and stimulation studies clearly demonstrate the crucial role the MEA plays in the expression of defensive behaviors.

The MEA has also been shown to be important for mediating the expression of reproductive behaviors. Mating or mere chemoinvestigation of reproductive stimuli, such as female urine or hamster vaginal secretion induced *c-fos* expression in MEApd in male rodents (Coolen et al., 1998; Heeb and Yahr, 1996; Newman et al., 1997). Our study (Choi et al, 2005) extended this finding and demonstrated that female urine induces increased number of *c-fos* expressing-neurons in MEApv as well, albeit not as many as in MEApd.

Consistent with the *c-fos* activation studies, broadly lesioning the MEA, including MEAa, MEApd, and MEApv produced deficits in mating. More importantly, damage to the MEA produced abrogation of both the chemoinvestigatory and copulatory phases of the mating (Dominguez et al., 2001; Heeb and Yahr, 2000; Kostarczyk, 1986; Newman et al., 1997). The effect was specific to the MEA because the lesioning of the basolateral amygdala failed to produce the same deficit in the reproductive behaviors (Kondo, 1992). In male gerbils, cytotoxic lesions specifically placed in the MEApd recapitulated the results of the whole MEA lesion and disrupted mating. Furthermore, the effect was not exacerbated by the damage to the MEAa (Heeb and Yahr, 2000). These results underline the crucial role of the MEApd in mediating reproductive behaviors. It is noteworthy to mention however, that contradictory results were obtained in male hamsters; damage to the MEAa, and not to the posterior MEA, abolished copulatory behaviors (Lehman and Winans, 1982; Lehman et al., 1980). It is not understood whether the disparity between the two results is due to the species-specific differences, extent of the damage to each area, or other unknown factors.

Finally, the MEApd also contains high densities of neurons that express estrogen receptors and androgen receptors (Simerly, 2002). Thus, the abundant functional and expression studies strongly suggest that the MEApd plays a unique role in modulating and controlling reproductive behaviors perhaps by integrating the hormonal status of the animals and the chemosensory input it receives from the AOB.

Hypothalamic medial behavior control column

The hypothalamus is responsible for coordinating autonomic, neuroendocrine, and somatormotor responses that guarantee homeostasis and are necessary for the survival of

individuals (Swanson, 1987). Furthermore, the hypothalamus is essential for the three basic classes of innate behaviors: ingestive, reproductive, and defensive behaviors. The fundamental evidence supporting this conclusion came from observing chronic midbrain and chronic hypothalamic animals.

Chronic midbrain animals, with a transaction of neuraxis between the forebrain and midbrain are completely aphasic, adipsic, cannot reproduce, and cannot mount defensive responses. On the other hand, chronic hypothalamic animals, which have an intact hypothalamus with the telencephalon and thalamus removed, can eat and drink, reproduce, and exhibit defensive behaviors (Grill and Norgren, 1978; Macht, 1958; Swanson, 2000). Combined with the observations that chronic hypothalamic animals are capable of generating spontaneous motor activities, whereas the chronic midbrain animals are not, the above results suggested that the hypothalamus contains motor pattern controllers for ingestive, reproductive, and defensive behaviors (Swanson, 2000).

These initial studies were followed by more finely-tuned functional studies (lesion and stimulation assays) and axonal projection studies. These studies indicated that there are two highly interconnected circuits within the medial column of the hypothalamus that respectively orchestrate the expressions of reproductive and defensive behaviors, two forms of innate social behaviors we are interested in. The anterior hypothalamic nucleus (AHN), the dorsomedial part of the ventromedial hypothalamic nucleus (VMHdm), and the dorsal premammillary nucleus are involved in the expression of defensive behaviors. On the other hand, the medial preoptic nucleus (MPN), the ventrolateral portion of the ventromedial hypothalamic nucleus (VMHvl), and the ventral premammillary nucleus (PMv) are

implicated in the reproductive behaviors (Canteras, 2002; Coolen et al., 1998; Kollack-Walker and Newman, 1997; Petrovich et al., 2001; Simerly, 2002; Swanson, 2000).

Electrical stimulation along various points within the hypothalamus indicated that defensive behaviors can be elicited from the AHN and the VMH. The VMH is divided into two subnuclei, dorsomedial (VMHdm) and vetnralateral (VMHvl), and some of the more detailed studies indicate that the VMHdm rather than the VMHvl is the neural substrate from which the behavioral effect can be induced (Fuchs et al., 1985; Kruk et al., 1983; Lammers et al., 1988; Maeda and Maki, 1989; Sweidan et al., 1991). Moreover, the behavioral responses electrically elicited from these sites resembled somatomotor and autonomic responses of animals facing natural threats, therefore suggesting that the AHN and the VMHdm modulate innate defensive responses under natural conditions (Canteras, 2002).

This notion has been tested by exposing rats to a live cat, a natural predator, or cat odor and examining *c-fos* expressions as a marker of neuronal activations. Consistent with the stimulation assays, direct exposure to the predator or simply predatory odor increased the *c-fos* expression in the AHN and the VMHdm. In addition to these two hypothalamic nuclei, the PMd also exhibited increased *c-fos* expression in response to cat odor (Canteras and Goto, 1999; Dielenberg et al., 2001; McGregor et al., 2004). Bilateral ibotenic acid lesions, which spare fibers of passage, placed in the PMd virtually eliminated defensive responses (escape and freezing) during the predatory encounter (Canteras et al., 1997), demonstrating that the PMd along with the AHN and the VMHdm form the distinct medial hypothalamic system critical for the expression of innate defensive behaviors.

Another body of work indicates that the MPN, VMHvl, and PMv participate in the circuit controlling the expressions of reproductive behaviors. MPN, VMHvl, and PMv, along with the MEA and BSTpr, are sexually dimorphic, and they contain a high density of hormone-responsive neurons (Gorski, 1984; Segovia and Guillamon, 1993; Simerly, 2002). Moreover, cells in these nuclei are activated, as assayed by the *c-fos* expression, when the animals are exposed to reproductive chemosensory stimuli or when they are allowed to fully mate (Bressler and Baum, 1996; Coolen et al., 1998; Heeb and Yahr, 1996; Kollack-Walker and Newman, 1997; Yokosuka et al., 1999).

Functional studies revealed that the MPN and the VMH control masculine and feminine sexual behaviors, respectively. The first indication of the MPN's involvement in male reproductive behavior was provided by the experiments, in which androgen implants in the MPN were found to restore masculine sexual behaviors in castrated rats and mice (Davidson, 1966; Matochik et al., 1994). Stimulation of the MPN enhanced male reproductive behaviors (Matochik et al., 1994; Rodriguez-Manzo et al., 2000). On the other hand, damage to the MPN, whether done electrolytically or excitotoxically, impaired them while leaving the female sexual behaviors intact. It is worthy to note that although the MPN lesion impaired the copulatory behaviors (mounting and intromission), it left the chemoinvestigatory phase of the mating and the ability to emit ultrasounds in response to female chemosensory stimuli intact (Bean et al., 1981; Klaric and Hendricks, 1986; Singer, 1968). These results are in marked contrast to the MEA lesions, which abolished both the chemoinvestigatory and copulatory phases of the mating, and suggest that the function of the MPN is concerned with the copulatory performance of masculine sexual behaviors rather than the arousal and appetitive behaviors reflective of sexual motivation (Swanson, 1987).

Contrary to the MPN, the VMH has been studied for its role in feminine sexual behaviors. Electrical stimulation of the nucleus facilitates the lordosis reflex (indicative of female sexual receptivity) (Pfaff and Sakuma, 1979b), while the lesion results in a deficit in the reflex (Malsbury et al., 1977; Pfaff and Sakuma, 1979a). Interestingly, both the stimulation and the lesion studies revealed that the lateral rather than the medial portion of the nucleus is the site controlling the lordosis reflex by facilitatory output.

Although its behavioral relevance is not clearly understood, activation of the PMv in males after exposure to the female soiled beddings (Yokosuka et al., 1999) and its interconnections with the MPN and the VMHvl suggest that it comprises of the hypothalamic circuits controlling expressions of reproductive behaviors (Simerly and Swanson, 1986). This interpretation is also consistent with the data showing that the pheromone-induced LH rise in the serum was completely prevented by a bilateral lesion that destroyed the PMv (Beltramino and Taleisnik, 1985). The intrahypothalamic projections of the PMv are unusual among the nuclei in the medial zone because they project exclusively to the periventricular zone nuclei (Canteras et al., 1992), which contain most of the neuroendocrine motoneurons (Swanson, 1987). Therefore, these lines of evidence suggest that the PMv may play a modulatory role in producing reproductive behavioral output in response to pheromonal signal by regulating gonadotropin release.

Bed Nucleus of Stir Terminalis (BST)

The BST is the region of telencephalon that lies ventral to the lateral septal nucleus and dorsal to the preoptic regions of the hypothalamus, and it surrounds the anterior commissure (Ju and Swanson, 1989). Its anterolateral group is implicated in maintaining

homeostasis based on its interconnections with the central amygdalar nucleus and with the various hypothalamic cell groups associated with the control of autonomic responses (Dong and Swanson, 2004a). On the other hand, several lines of evidence suggest that two subnuclei within the posterior division of the BST, the BSTpr and the BSTif regulate the expressions of reproductive and defensive behaviors, respectively.

Mating and inter-male aggression activated *c-fos* expressions in the BSTpr and the BSTif. More importantly, the expression of *c-fos* in the BSTpr was specific to mating and was not the result of general social interactions, as the aggressive behaviors failed to produce *c-fos* expression in the subnucleus (Coolen et al., 1998; Kollack-Walker and Newman, 1995). The lesion studies were consistent with the neuronal activation studies; a large lesion covering the entire BST, or damage confined to the BSTpr, produced a deficit in copulatory behaviors. In addition, these animals showed decrements in the chemoinvestigatory behaviors and in sexual arousal inferred from the non-contact erection (Claro et al., 1995; Liu et al., 1997; Powers et al., 1987). The foregoing data combined with the observation that the BSTpr is a sexually dimorphic nucleus containing many hormone-responsive neurons, imply that this nucleus participates in the circuit controlling the mammalian reproductive behaviors.

Electrical stimulation of the posterior BST also induces aggressive behaviors (Shaikh et al., 1986). Although there is no specific functional study carried out to gain more insight into the specific role of the BSTif in regulating defensive behaviors, its connections with other defense-related nuclei in the MEA and the hypothalamus (see below in the section 'Projections'), and its neuronal activation under social stress (Kollack-Walker and Newman 1995), highly suggest that it constitutes the amygdalar-hypothalamic defensive circuitry.

In conclusion, *c-fos* neuronal activation studies, lesion studies, and electrical stimulation assays indicate that the highly interconnected group of hypothalamic nuclei MPN, VMHvl, and PMv along with MEApd and BSTpr, are involved in modulating reproductive behaviors. Their counterparts, AHN, VMHdm and PMd (these form another set of interconnected circuit in the hypothalamus), with MEApd and BSTif, participate in a circuit involved in the expressions of defensive behaviors. It is striking that at each node within the circuit, there is a reproductive and defensive counterpart located adjacent to each other: MEApd versus MEApv, BSTpr versus BSTif, VMHdm versus VMHvl, and PMd versus PMv. It raises the question of whether the circuit components organized in this way have a functional significance in controlling two opponent behaviors of reproduction and defense (see below 'Labeled Line and Behavioral Antagonism').

Projections

It is not so surprising that there is a direct connection from the MEA, which receives the chemosensory information, to the nuclei in the hypothalamic medial behavior control column. What is perhaps the most interesting is that these connections are topographically organized and segregated in a striking manner (Petrovich et al., 2001; Swanson, 2000).

The MEApd, which is considered to be a reproductive portion of the medial amygdala, projects only to the hypothalamic nuclei associated with expressions of reproductive behaviors: MPN, VMHvl, and PMv. On the other hand, the MEApv and MEAa project to

both the reproductive hypothalamic nuclei and their defensive counterparts: AHN, VMHdm, and PMv (Canteras et al., 1995; Petrovich et al., 2001; Simmons and Yahr, 2002) (Figure 2).

In addition to the direct connection, the MEA projects indirectly to the hypothalamic nuclei through the posterior BST, and these projections are also topographically organized (Dong et al., 2001; Dong and Swanson, 2004b; Gu et al., 2003). The BSTpr, a reproductive portion, receives inputs from the MEApd and in return projects to the reproductive hypothalamic nuclei. On the other hand, the BSTif, which receives input from the MEApv/MEAa, projects to the defensive hypothalamic nuclei.

There are a few exceptions to this pattern of segregated circuits from the MEA to the BST, and from the BST to the hypothalamic nuclei, or from the MEA directly to the hypothalamic nuclei involved in reproduction and defense. First, the terminals from the MEApv/MEAa are not confined to the defensive portion of the BST (BSTif), but they are also found in its reproductive counterpart, the BSTpr (Dong et al., 2001). Thus, the MEApv/MEAa projects to the defensive nuclei and to the reproductive nuclei both in the BST and the hypothalamus. Secondly, the terminals from both the BSTpr and the BSTif are observed in the PMv, a reproductive hypothalamic nucleus (Dong and Swanson, 2004b). Thirdly, there is an atypical connection from a reproductive hypothalamic nucleus, the VMHvl, to a defensive hypothalamic nucleus, AHN (Canteras et al., 1994).

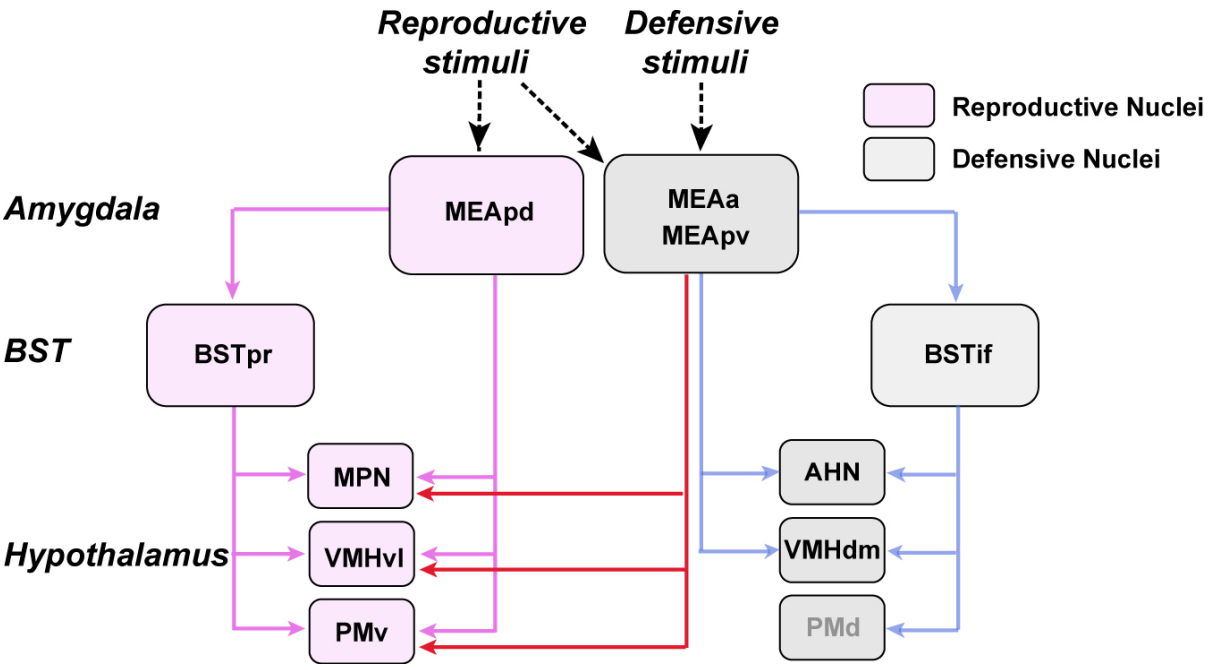

Figure 2. Amygdalar-hypothalamic projections.

MEApd is activated by reproductive stimuli and projects to the reproductive nuclei of the posterior BST and the hypothalamus. On the other hand, MEAa/MEApv is activated by both reproductive and defensive stimuli and projects to both the reproductive and defensive nuclei of the BST and hypothalamus.

Outstanding Issues

Genetic determination of the circuits

The amygdalar-hypothalamic circuits for reproduction and defense are topographically organized and anatomically segregated. Such stereotypically arranged circuit models suggest that it is genetically determined during embryonic development.

There is a precedent for genes specifying axonal projection patterns of distinct subpopulations of neurons. In vertebrates, motoneurons in the ventral spinal cord are arranged into columns (subtypes of motoneurons), and each column of neurons project to different target muscles. Both gain-of-function and loss-of-function studies indicate that the combinatorial expression of LIM homeodomain genes is responsible for conferring motoneuron subtypes with their target specificity (Kendel, 2000; Shirasaki and Pfaff, 2002).

In the same manner, the projection specificity of MEA neurons to either reproductive or defensive nuclei in the hypothalamus must be determined by differential gene expressions. In order to search for such genes, we compared the gene expression profile of the MEApd to that of the MEApv using oligonucleotide microarrays in adults and also by examining ~100 candidate genes by *in situ* hybridization at embryonic day 14.5.

Functional significance of the asymmetric projection patterns of MEApd versus MEApv

Although the projections from the MEA to the hypothalamic behavior control column (either directly or indirectly through the BST) are strikingly segregated, there are a few exceptions to this trend, and most of these exceptions are asymmetrically allocated to the defensive portion of the circuitries.

The MEApv, which is mainly activated by defensive olfactory stimuli, projects to the reproductive as well as to the defensive nuclei of the hypothalamus. Axons originating from the MEApv also terminate in the reproductive portion of the BST (BSTpr) as well as in the defensive portion (BSTif). The BSTif, in turn, projects to the PMv (reproductive nucleus) in addition to the defensive hypothalamic nuclei. Thus, all the reproductive nuclei of the hypothalamus receive dual inputs: one originating from the MEApd and BSTpr (reproductive), and the other originating from the MEApv and BSTif (defensive). BSTpr, likewise, gets inputs from both the MEApd and MEApv. I tried to uncover the functional significance of this asymmetry and the resulting dual inputs to the reproductive nuclei in my work.

Labeled Line and Behavioral Antagonism

Many studies to date suggest that distinctive chemosensory stimuli detected by the narrowly-tuned VNO are relayed to the AOB mitral cells without much transformation of the data (refer to the section "Vomeronasal Organ"). The chemosensory information is subsequently transmitted to the dedicated amygdalar-hypothalamic circuitries, one for the expression of reproductive behaviors and the other for the defensive behaviors with very little interaction between the two. Thus, with the exception of the connection from the AOB to the MEA, details of which are still unknown, the input all the way from the VNO to the central pattern controllers in the medial hypothalamus seems to follow labeled lines without much processing of the incoming information.

If an animal is faced with only a single stimulus at a given time, then the labeled line model appears to be sufficient. However, they are frequently faced with conflicting cues in

their natural environment. Moreover, expressions of reproductive and defensive behaviors are mutually exclusive. Animals typically do not engage in reproductive behavior while being faced with threatening stimuli. Therefore, the parallel, labeled-line circuit organization for the two antagonistic behaviors pose a problem for animals, which must make rapid decisions to engage in defensive versus reproductive behaviors when they are faced with conflicting signals (Lima and Dill, 1990).

The same problem is apparent in the gustatory system. In mice, it has been found that there are dedicated receptors and cells for detecting either sweet or bitter tastants and for generating their respective behavioral responses: ingestion for sweet tastants and avoidance for bitter tastans (Scott, 2004; Zhang et al., 2003; Zhao et al., 2003). Moreover, ectopically expressing a novel receptor, unrelated to the taste-system, in sweet-detecting cells and inducing ingestion upon activating this heterologous receptor, strongly implicated that there are dedicated circuits not only for detecting the sweet versus bitter tastants, but also for generating subsequent behaviors appropriate for each tastant (Zhao et al., 2003). However, similar to the reproductive versus defensive behavioral system, animals frequently encounter both sweet and bitter tastants and must make clear-cut decisions between competing ingestive versus avoidance behaviors. Sometimes individual compounds, such as artificial sweetners, activate both sweet and bitter tasting cells; presumably upon activation of both circuits, however, animals decide to ingest the substance rather than to avoid it. Therefore, depending on the results of the cost-benefit calculations animals have made, they would choose to consume even bitter and sometimes potentially toxic substances (Gillette et al., 2000). In order for such a calculation to be made, there must be cross-talk between two

circuits, and there must be a neural substrate in which the strength and quality of two conflicting cues are integrated to generate the sum result as motor output.

In the same manner, decision-making between the reproductive and defensive behaviors would seem to require cross-talk between the two sub-circuits. However there are very few interconnections between the reproductive and defensive hypothalamic nuclei. The MEApv projects to the reproductive as well as the defensive hypothalamic nuclei, but the function of this divergent projection is not known. Some evidence suggests that suppression of reproductive behaviors by threatening stimuli may be exerted within the amygdalar-hypothalamic pathway. For example, exposure of virgin female rats to newborn pups promotes defensive behaviors and inhibits maternal behavior (Numan and Sheehan, 1997; Sheehan et al., 2000). This inhibition can be overcome by lesions of the medial amygdala, and involves projections from this structure to VMH (Sheehan et al., 2001). The circuit-level mechanisms that mediate such behavioral inhibition, however, are not understood.

In the course of my study, I have come across an unexpected circuit node within the amygdalar-hypothalamic circuits that may serve to "gate" reproductive behaviors by defensive stimuli. In the following chapter, I propose a testable model of how the cross-talk between the two sub-circuits of reproduction and defense within this neural substrate may function to resolve behavior antagonism when two competing stimuli are present.

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