CHAPTER 1
Preclinical Research and Animal Models in Sexual Medicine

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Introduction

Preclinical research typically involves the use of animal models of human sexual response, and is often conducted to investigate the effects of pharmacologic agents, instrumentation, new devices, or surgical procedures prior to clinical trials. This research may also examine certain side effects of such treatment; however, preclinical research may also include human tissue experiments or biochemical experiments with human products, e.g. native or recombinant enzymes. For the sake of simplicity, studies of toxicology, carcinogeniticity, fertility, and safety will not be included in the definition.

The key issue for clinicians is the ability to extrapolate the preclinical results to human clinical populations, and in particular to determine the likelihood that a treatment will be successful or will warrant subsequent human tests. Besides studies conducted in anesthetized animals that have been extremely useful in the study of sexual physiology, behavioral experiments are crucial to providing a more integrative approach to understanding the physiologic and pathophysiologic aspects of sexual function and dysfunction.

In all species, sexual behavior is directed by a complex interplay between steroid hormone actions in the brain that give rise to sexual arousability, and experience with sexual reward or pleasure that gives rise to expectations of competent sexual activity, including sexual arousal, desire, and performance. Sexual experience allows animals to form instrumental and Pavlovian associations that predict sexual outcome and thereby direct the strength of sexual responding. Although the study of animal sexual behavior by neuroendocrinologists has traditionally been concerned with mechanisms of copulatory responding; more recent use of conditioning and preference paradigms, and a focus on environmental circumstances and experience, has revealed behaviors and processes that resemble human sexual responses.

Accordingly, we have summarized behavioral paradigms used with rodents and other species that are analogous or homologous to human sexual arousal, desire, reward, and inhibition. At a superficial level, human copulatory behavior does not resemble copulatory behavior in animals. For example, there is no human counterpart to female rat lordosis (at least not as an unambiguous, estrogen-dependent postural display of sexual receptivity in females), and human sexual behavior is so shaped by experience and learning that it seems to defy hormone actions that are critical to the display of animal sexual behavior. However, insights into the human experience can indeed be derived from animals, and in ways that are far less difficult scientifically and ethically to obtain than from human populations.

We have not referenced any experimental techniques because this was far beyond the scope of this chapter. Instead, we have proposed a list of review papers that will provide the reader with more in-depth insight into different experimental models.

What is required for a good animal model?

Predictive validity is the most important requisite of an appropriate animal model. In addition to this,

Penile erection
Recording of intracavernous pressure increases in anesthetized or conscious animals
Penile reflex tests
Ex copula erections
In vitro studies of cavernosal strips / penile artery reactivity (organ baths)
Cavernosal smooth muscle cells culture
Biochemical studies of erectile tissue
Ejaculation
Mating test: latency to ejaculate
Urethrogenital reflex (anesthetized)
PCA-induced ejaculation (anesthetized or conscious)
Pudendal motoneuron reflex discharge (anesthetized)
Electrical stimulation of peripheral nerves (anesthetized)
La Peyronie's disease
TGF-β1-induced La Peyronie's like condition
Priapism
Rabbits exposed to corporal hypoxia, then penile erection elicited by neural stimulation and the base of the erect penis clamped
eNOS–/– and nNOS–/–, eNOS–/– mice
Female peripheral sexual arousal
Anesthetized dogs, rabbits and rats: vaginal vasculo-muscular response along with clitoral tumescence induced by peripheral electrical neural stimulation.

Table 1.1 Experimental paradigms that can be used as rodent models of human sexual functions. eNOS, endothelial nitric oxide synthase; nNOS, neural nitric oxide synthase; PCA, p-chloroamphetamine; TGF-β1, transforming growth factor beta 1.

animal models should be simple and practical enough to have "high throughput", meaning the ability to have experiments conducted relatively quickly. Issues of sample size and ease of testing and analysis are key factors. The validity of any homologous or analogous animal model can only be determined in situations that test whether a treatment that modifies behavior in the animal does so in humans.

Any animal "system" in which the homology or analogy has predictive validity to human responses or physiologic processes (and can be replicated) is a good model. If the model is practical from an experimental standpoint, then it will likely be used more than models that are cumbersome. In addition, the more information that is gathered from a particular model, the more the model will be used because it has a large literature associated with it. Rats continue to be the most frequently used animals in the study of sexual behavior. There are many reasons for this, the most obvious being that they are practical (e.g. small, easy to handle, and quite social) and they have a large literature associated with them. Rats also resemble humans in many analogous and homologous ways. Certain tissues and neuroendocrine systems in rats are strikingly similar to our own (e.g. the physiologic control of erection, or uterine tissue growth following estrogen treatment).

Rectification of terms

In humans, sexual dysfunctions form around the categories of sexual arousal, desire, orgasm, and pain. Arousal may be separated into physiologic genital arousal (sometimes referred to as "potency") and subjective or psychologic arousal that denotes a conscious awareness of the genital sensations. However, this psychologic arousal may be an important component of sexual desire (sometimes referred to as "libido" or "motivation"). Sexual arousal and desire sum into behavioral responses of copulation

Table 1.2 Paradigms that can be used as rodent models of human sexual behavior (from Pfaus *et al.,* 2003).

Sexual arousal
Males
- Penile reflex tests (physiologic erectile function; responses to somatosensory stimulation)
- Noncontact erections ("psychogenic" erectile function; responses to primary or secondary conditioned sexual cues)
- Copulatory measures: latency to mount, intromit or ejaculate (shorter latency = greater arousal)
- Enforced interval effect (model of premature ejaculation)
- Coolidge effect (increased arousal by changing sexual stimuli)

Sexual desire
Females and males
- Excitement (motor responses in anticipation of sexual activity or in response to hormonal stimulation)
- Instrumental responding (desire to obtain a sex partner)
- Sexual preference paradigms (desire to obtain unconditioned or conditioned sexual incentive characteristics)
- Copulatory measures

Males
- Pursuit (desire to obtain sex partner)

Females
- Solicitation, hops and darts (desire to initiate sexual activity)
- Pacing (desire to regulate copulatory contact; increased pacing = decreased desire for copulatory contact)
- Lordosis (receptivity to vaginal penetration)
- Lateral tail displacement in hamsters (receptivity to vaginal penetration)

Sexual reward
Females and males (to examine what aspect of sexual responding is rewarding, e.g. copulatory stimulation vs. ejaculation in males, or the ability to control sexual interaction in females):
- Operant responding for primary or secondary sexual reinforcers
- Conditioned place preference
- Unconditioned or conditioned partner preference
- Conditioned copulatory behavior (e.g. copulatory responses in places paired with sexual or other rewards, or in the presence or absence of conditioned incentive cues)

Sexual inhibition
Females
- Copulatory behavior after several ejaculatory series
- Estrus termination
- Tests using ovariectomized females primed with estrogen alone

Males
- Primary sexual inhibition (using access to nonreceptive females)
- Second order sexual inhibition (using odors or other stimuli paired with access to nonreceptive females).
- Recovery from sexual exhaustion

(sometimes referred to as "performance"). The terms used in the animal literature often do not resemble those in the human literature, and a rectification of terms is necessary to translate between animal and human sexual functions. We propose the Incentive Sequence Model (Fig. 1.1) as a place to begin such a rethinking of nomenclature and to bridge the gap between animal research and the clinical practice.

Male Sexual Function

Peripheral sexual reflexes (erection and ejaculation), copulatory behaviour (mounts, intromissions,

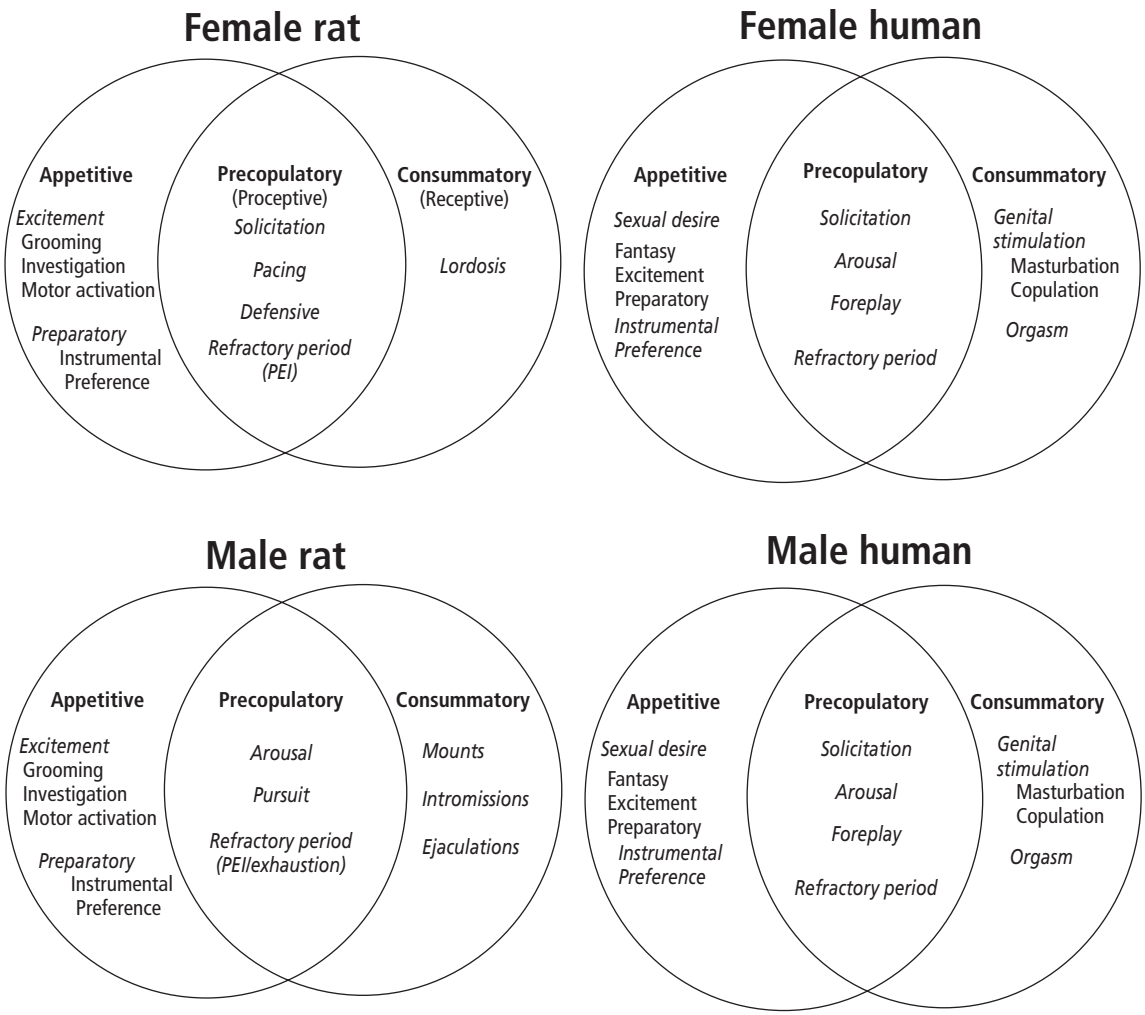

Fig. 1.1 Incentive sequences for human and rat sexual behaviors. This model provides a conceptual way to denote classes of homologous or analogous behaviors between the species (and sexes). The behavioral stream moves from left to right, through appetitive, precopulatory, and consummatory phases of behavior. This conforms to the movement of animals from distal to proximal to interactive with respect to the sexual incentive. Three types of appetitive responding reflect relative degrees of learning and necessity. "Preparatory" behaviors are learned responses that animals must make in order to acquire the incentive (e.g. operant behaviors, pursuit, etc.). "Anticipatory" behaviors are learned responses that occur in anticipation of an incentive, but are not necessary to obtain it (e.g. conditioned psychomotor stimulation that characterizes behavioral excitement). Unlearned appetitive responses also exist that are instinctual (e.g. unconditioned anogenital investigation). These aspects of behavior also occur once copulatory contact has been made, especially if copulation occurs in bouts (as it does in rats). From Pfaus, 1999.

and ejaculation), and appetitive conditioned sexual responses (e.g. conditioned arousal), have been examined in a variety of species. In most cases, sexual physiologic and behavioural responses are extremely similar between the species, making the generation of analogies and homologies, and their application to human male function and dysfunction, straightforward.

Penile erection

Experimental research on penile erection dates from at least the 19th century, with the work of pioneer physiologists such as Eckhardt, Langley and Anderson. Subsequently, during the 20th century significant advances were achieved thanks to the work of Semans and Langworthy in the 1930s, veterinary researchers performing experiments in conscious bulls and stallions in the 1970s, Sjöstrand using plethysmography to quantify penile erection in the rabbit, and then work by Lue's and Goldstein's groups in the 1980s, providing the scientific and medical community with experiments conducted in dogs, monkeys and rabbits that show the vascular component of penile erection and the crucial role of cavernosal smooth muscle fibers. Quinlan then introduced the first rat model to measure penile erection. More recently, investigations of penile erection have been performed in mice, opening the door to studies conducted with genetically modified animals.

From a physiologic perspective, it appears that there is a close similarity between local mechanisms of penile erection between non-human mammals and human males except for the role of striated muscles, which are less important in humans compared to various animal species.

Evaluation of erectile response in anesthetized animals

The gold standard for quantitative measurement of penile erection during experiments conducted in anesthetized and conscious animals is the recording of intracavernous pressure (ICP), also measurable in conscious animals by telemetry. It is noteworthy that ICP is closely dependant from arterial blood pressure. Penile erection can be elicited in anesthetized animals by electrical stimulation of peripheral nerves, i.e. pelvic or cavernous nerves (Fig. 1.2). It can also be elicited by electrical or chemical stimulation applied to brain structures. Drug delivery everywhere in the periphery (from intracavernosal injections to oral gavage) or within the central nervous system, including brain and spinal cord, is feasible in anesthetized animals to study their effect on penile erection.

Animal models have been widely used to establish the effects of phosphodiesterase-5 (PDE-5) inhibitors, and they have been predictive for the human situation for this class of compounds. There are crucial questions to be answered before extrapolating experimental data to humans, including: is the

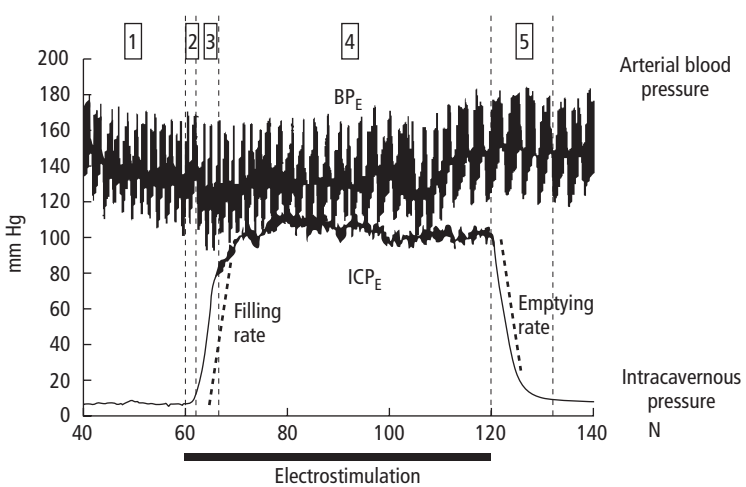

Fig. 1.2 Computer tracing of original recording of intracavernous and arterial blood pressures when stimulating cavernous nerve (6 volts, 10 Hz. 1 millisecond). 1: flaccid state; 2: latent phase; 3: tumescence phase; 4: maximal tumescence of the corpora cavernosa; 5: detumescence phase. ICP_E and BP_E were mean values of intracavernous and arterial blood pressures during full phase. Filling rate of corpora cavernosa is slope of increase of intracavernous pressure during tumescence phase. Slope of dramatic drop of intracavernous pressure after end of stimulation represents corpora cavernosa emptying rate.

receptor targeted by the compound under investigation the same in animals as compared to the human male and does it play the same role; e.g. intracavernosal prostaglandin E_1(PGE_1) injections do not elicit penile erection in many animal species. When investigating the proerectile effect of pharmacologic compounds in animals, the following aspects must always be taken into account: dose used, route of administration, pharmacokinetics, half-life, metabolism of the studied compounds, etc.

In vitro reactivity studies of erectile tissue

These experiments are conducted in the investigation of the local cellular mechanisms of penile erection. Cavernosal strips from various animal species and humans have been studied in organ baths. These tissues are either pharmacologically precontracted, e.g. with phenylephrine, or electrically stimulated (electrical field stimulation) to elicit the release of neurotransmitters contained within the nerve terminals present in the tissue. Numerous compounds (e.g. prostanoids, α-adrenoceptor blockers, endothelin antagonists, PDE-5 inhibitors) have been found to be able to relax cavernosal strips by targeting various intracellular systems. Primary cell cultures derived from animal or human corpus cavernosum have also been used as *in vitro* models to define cellular mechanisms involved in erectile function.

Pathophysiologic models of erectile dysfunction

A wide variety of pathophysiologic models of erectile dysfunction (ED) have been proposed, aiming to mimic the numerous pathologic conditions responsible for ED in clinical practice. A non-exhaustive list of these models comprises: hypertensive rats, atherosclerotic rabbits, diabetic rats and rabbits, aged rats, castrated rats, cavernous nerve-injured rats, alcohol-treated, or nicotine-treated rats. The question of extrapolation to humans using these various experimental conditions must always be asked before drawing conclusions regarding applicability. It is noteworthy that there is no established standard in this domain, therefore we propose that the endpoints must be analogous or homologous (e.g. the restoration of erectile capability sufficient for copulation).

A special caution must be paid to castrated animals: many conclusions regarding the role of testosterone on penile erection have been drawn from experiments conducted in castrated rats; unfortunately this experimental paradigm is very different from the human situation, which is highly prevalent—i.e. the ageing male with partial androgen deficiency syndrome due to age in males (PADAM). So far no reliable model of PADAM has been proposed.

Due to advances in molecular biology, genetically engineered mice have now become available. Although the ultimate goal of these models is to develop gene therapy to rectify gene activity, transgenic or knockout mice have recently contributed to our understanding of the physiologic mechanisms of erectile function, as well as to various pathophysiologic processes occurring during ED.

Study of erection in conscious animals

In conscious rats, penile erections can be studied in isolation to obtain measures of physiologic arousal, or in response to different types of sexually arousing stimuli (e.g. noncontact erections in response to estrous odors) to obtain measures of psychogenic arousal.

Ex copula reflex erection is a commonly used unanesthetized rat model of erection. The rat is lightly restrained in a supine position and the penis is retracted from the sheath. Relatively predictable "spontaneous" penile erections are thus elicited. *Ex copula* reflex erections are generated by spinal reflex mechanisms and modulated by supraspinal control. The effects of drugs and various central neural lesions can be examined in this model. It has the advantage in that it does not involve social interaction with the female and it examines penile reactions directly.

Several pharmacologic agents acting at different central brain regions have been shown to elicit penile erection in conscious rats during *ex copula* penile erection tests, i.e. in isolation. Penile erection during these tests has been inferred from a series of motor acts like standing on the hind limbs, the head of the animal oriented towards the genital area, licking of the genital area, contractions of the hip muscles, and sometimes by direct observation of protrusion of the glans. It remains unknown whether or not the putative neural structures representing targets for these "proerectile drugs" are all activated during the

penile erection that occurs in several natural contexts (sleep, copulation, psychogenic, reflex).

Male rats (but only pigmented strains) also show an analogy of "psychogenic erection" in response to the presence of estrous females, even when physical contact is prevented. A model of noncontact erections (NCEs) in rats was developed by Sachs and colleagues, and is studied in the presence of an inaccessible receptive female behind a mesh screen, or behind a series of walls with an air circulation system that brings the estrous odors to the male's compartment. Erections in response to these salient sexual cues are viewed as a model of psychogenic erection because they do not require direct somatosensory stimulation to be induced. The neurochemical and hormonal mechanisms that control their expression have been studied in detail. Analysis of NCEs following discrete brain lesions has demonstrated important distinctions in the neural control of copulatory performance and erectile capability. Drugs that enhance noncontact erections also induce a "penile erection and yawning syndrome" in rats in the absence of sexual stimulation. NCEs offer at least one advantage over the study of erection during copulation: they do not require complex motor responses or direct social interaction. This makes their study relatively less ambiguous as a measure of subjective sexual arousal. One concern is that the dependence of these responses on olfactory stimuli is unlike human sexual responses, which are more dependent on visual and auditory cues. However, olfaction is analogous to vision in this case, as the former is the dominant sense in rodents, whereas the latter is the dominant sense in humans.

Conclusions

Experimental research has been very productive regarding the physiology, pathophysiology and pharmacology of penile erection. There exist several rodent models of penile erection, from higher neural control down to molecular events within the erectile tissue. Although care must always be taken before extrapolating quickly from experimental data to the clinical situation, there is a high degree of predictability from rat models to men. Nature appears to have conserved mechanisms of erection in mammalian males.

Ejaculation and male orgasm

Most of the experimental work done so far for the investigation of ejaculation is based on behavioral experiments. Ejaculations in rats can be studied much the same way they are studied in humans, with the latency from first mount or intromission to ejaculation being the key variables (Fig. 1.3). Male rats typically ejaculate following several penile intromissions, and can ejaculate several times before becoming sexually exhausted, in which the male no longer responds to estrous odors or female solicitations. During successive ejaculatory series, the refractory period or post ejaculatory interval between each ejaculation and the subsequent resumption of copulation increases progressively. Penile intromission requires erection, and ejaculation typically requires sensory feedback from the penis that accumulates with multiple intromissions. The number of intromissions before ejaculation, the number of ejaculations achieved in a timed test, and the length of the post ejaculatory interval, are all dependent on autonomic arousal and can be enhanced or disrupted by drugs that have similar effects on copulatory performance in men. For example, drugs that delay or abolish orgasm in men (e.g. selective serotonin reuptake inhibitors such as fluoxetine, paroxetine), increase the ejaculation latencies and reduce the total number of ejaculations in rats. As in men, the reduced ability to ejaculate is more pronounced in rats following long-term daily administration. Acute alcohol intoxication also delays ejaculation in men and male rats.

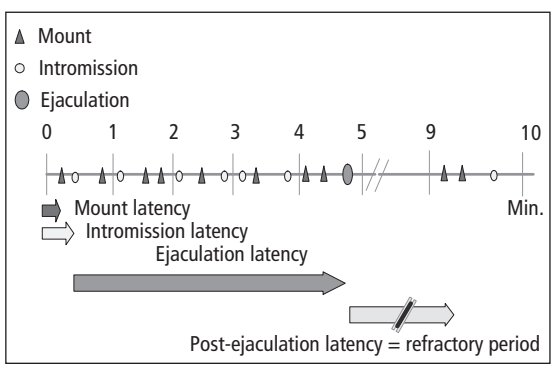

Fig. 1.3 Typical copulatory pattern in the male rat over a 10-min period.

Experimental investigation of ejaculation in anesthetized rats

Compared to penile erection much less research has been conducted in anesthetized animals in order to elucidate the physiology and the pharmacology of ejaculation. However, four interesting paradigms have been developed:

1 *Urethrogenital reflex:* the urethrogenital (UG) reflex is elicited by mechanical stimulation of the urethra in anesthetized and spinalized rat. Such a stimulation causes a spinal reflex to occur that consists of rhythmic contraction of the bulbospongiosus (BS) and the ischiocavernosus (IC) striated muscles associated with penile erection. It may be considered as mimicking the expulsion phase of ejaculation.

2 *PCA-induced ejaculation:* A model of pharmacologic induced ejaculation; p-chloroamphetamine (PCA) is an amphetamine derivative that liberates catecholamines and serotonin (5-hydroxytrptamine, 5-HT) from monoaminergic nerve terminals. Systemic administration of PCA has been reported to induce ejaculation in both conscious and anesthetized rats. Pharmacologic data indicate that the primary role in mediating the effect of PCA on ejaculation involves 5-HT, whereas noradrenaline (NA) appears to be of secondary importance.

3 *Electrical stimulation of peripheral nerves and ejaculation*: in anesthetized rats, electrical stimulation of the hypogastric nerve can partially reproduce the ejaculatory process, i.e. eliciting a rise in seminal vesicle and bladder neck pressures that correspond respectively to seminal vesicles contractions and closure of the bladder neck, but it fails to induce the expulsion reflex.

4 *Pudendal motoneuron reflex discharge*: the recording of electrical activity in the efferent branch of the ejaculatory reflex, i.e. the pudendal motor response discharge (PMRD) elicited by the electrical stimulation of the afferent branch of the expulsion reflex—the dorsal nerve of the penis, is thought to be an experimental model representing events that occur in humans during sexual intercourse and that culminate with the expulsion of sperm.

Orgasm and the consequences of ejaculation

Although it is not known whether male rats experience orgasm during ejaculation, the peripheral reflexes appear very similar. Moreover, ejaculation is absolutely necessary for male rats to show subsequent evidence that sex was rewarding or "pleasurable". For example, ejaculation is required for the induction of conditioned place and partner preference in male rats. These preferences are not displayed if males are administered the opioid antagonist naloxone during conditioning, suggesting that ejaculation induces the activation of endogenous opioids in the brain that mediate the critical rewarding properties of sex.

Conclusions

This research is still in its infancy. Apart from behavioral studies, there is a need for standardization and more research is definitely mandatory in this area. There is no model available, for example, to investigate delayed or absent ejaculation or painful ejaculation, and the experimental equivalent of the male orgasm is lacking.

Sexual motivation and "desire"

Desire has always been difficult to define objectively. In the DSM-IV-TR, the diagnosis of hypoactive sexual desire disorder is given when "desire for and fantasy about sexual activity are chronically or recurrently deficient or absent." By converse logic, then sexual desire is the presence of desire for, and fantasy about, sexual activity. This definition appears coherent but is circular. How does desire manifest itself?

Like people, animals manifest sexual excitement behaviorally. They increase their motor output in anticipation of copulation and work for the opportunity to copulate or to obtain primary or secondary (conditioned) sexual rewards associated with copulation. Animals will also choose between two or more sexual incentives based on the strength of the incentive cues and the animal's own internal drive state. What characterizes these behaviors is that they occur before copulation: Courtship, operant responses, conditioned locomotion in anticipation of sex, time spent near a particular sexual incentive, or choices made between two or more incentives, can all be considered analogies of anticipatory sexual desire. The strength of the behavior can be observed as increases or decreases, or can be tested by increasing the crite-

rion level of responding that animals must attain before they are given access to rewards. Simply put, animals with more "desire" will display more robust behavior than animals with less desire. Desire can also be inferred from certain appetitive responses that occur during copulation, such as solicitation in females or chasing behavior in males. A growing body of evidence indicates that these aspects of sexual behavior are altered in a relatively selective fashion by certain drugs that are known to alter desire in humans (e.g. by drugs that affect dopamine or melanocortin receptors).

Models of male sexual dysfunctions

Erectile dysfunction

Male rats that do not perform sexually are typically taken out of behavioral studies, so there is very little known about their actual erectile responsiveness. This proportion is generally low, especially if the males are pre-exposed to the test chambers prior to their initial sexual experiences. Some of these males do not display any interest in the female, and do not initiate any kind of sexual activity. However, other males display sexual interest and mount repeatedly, but do not achieve vaginal intromission. The lack of intromission may stem from an inability to achieve erection. Indeed, erectile responses in isolation and intromissions during copulation are both very sensitive to disruption by several classes of drug, including psychomotor stimulants, dopamine and noradrenergic antagonists, and opioid agonists. Acute or chronic treatment with selective serotonin reuptake inhibitors (SSRIs) does not appear to alter erectile responses or the number of intromissions prior to ejaculation. This profile of pharmacologic sensitivity is strikingly similar to clinical observations and anecdotes in men, thus male rats may be a useful model of drug-induced, if not also stress or vascular disease–related erectile dysfunction.

Premature ejaculation

In 1956, Knut Larsson published a series of studies that described many ways in which sexual reflexes and behaviors could be conditioned by experience. In one of these paradigms, called the "enforced interval effect", male rats were given repeated access to females that were removed physically from the testing chamber by the experimenter after every intromission. In this way, Larsson was able to vary the time between intromissions and found that male intromission intervals that lasted longer than normal resulted in males that learned to ejaculate with far fewer intromissions. One of the interpretations of this data was that the imposition of longer intromission intervals made males more sympathetically aroused, and led to either a faster ejaculation or one that required less tactile penile stimulation. This model of hyperstimulation of sympathetic outflow by either highly stimulating, unpredictable, or stressful sex, formed part of the basis of Masters and Johnson's model of premature ejaculation a decade later. Despite this, the model has not been developed further, nor has it been used widely to examine drug effects.

More recently it was reported that natural differences are found in the ejaculation latencies of male rats, which may indicate a more biological explanation of premature ejaculation that shares some of the characteristics of human premature ejaculation. In pooled populations of male albino Wistar rats during a 30 min standardized mating test, three categories of males were identified: (1) males that displayed a low number of ejaculations (0 to1) and were considered as sexually "sluggish" or "hypo-sexual"; (2) a second category of rats that showed a range of two or three ejaculations and were considered as "normal" ejaculators; and (3) males who displayed four or five ejaculations and were considered as "rapid" ejaculators or "hyper-sexual" rats. The number of ejaculations across the various studies was distributed according to a Gaussian curve: on one side approximately 10% of rats display "hypo-sexual" behavior and on the other side 10% display "hyper-sexual" behavior after at least four successive weekly sexual tests of 30 min. Interesting differences were found between the "sluggish" and "rapid" groups of rats with regard to a variety of other parameters of sexual behavior, resembling clinical symptoms of men suffering from retarded and premature ejaculation, respectively.

The "hyper-sexual" animals have been further investigated in order to know whether they could be used as a model for human premature ejaculation. Compared to "normal" ejaculators, ejaculation

latency was shorter in "rapid" ejaculators and longer in "sluggish" ejaculators. Intromission and mount frequencies, the latter being considered as a putative index of sexual motivation, did not differ between the three categories of ejaculators, suggesting no differences in appetitive components of sexual behavior. When investigating the effects of 8-hydroxy-2-di-n-propylamino-tetralin (8-OH-DPAT) in these three categories of males, this compound was shown to induce a statistically significant increase in the number of ejaculations displayed by "sluggish" and "normal" rats, and to decrease in a statistically significant way the ejaculation latency in the "sluggish", "normal" and "rapid" rats.

This experimental paradigm, despite the fact it has been recently reported and not yet confirmed by others, appears as extremely attractive. Indeed "hyper-sexual rats" likely represent a pathophysiologic model of premature ejaculation. It remains to be seen whether drugs that can counteract premature ejaculation in men can increase the latency of male rats to ejaculate in this condition. It also remains to be studied whether such males are uniquely susceptible to an "enforced interval effect".

Delayed ejaculation

Conversely, some male rats ejaculate infrequently, or take a long time to achieve ejaculation. As mentioned above, this can be induced pharmacologically with alcohol or chronic treatment with SSRIs, such as fluoxetine or paroxetine. It remains to be determined whether the subset of sexually "sluggish" male rats found in normal populations can be utilized as models of delayed ejaculation.

Hypoactive sexual desire

Some male rats do not copulate despite extreme attempts on the part of sexually receptive females to get them to do so (including repeated mounting of the male by the female). Some of these males can be stimulated to copulate with low levels of electric shock, tail pinch, or treatment with low doses of psychomotor stimulants, like amphetamine, suggesting that a more general hypoarousability mediates their lack of responsiveness. Stress may also induce hyposexual responsivity in male rats, especially if it is present during their early sexual experience. For example, a sizable proportion (often nearly 50%) of male rats will not copulate during their first trial with sexually receptive females unless they are pre-exposed to the testing chamber. Indeed, a large proportion of these noncopulators will *never* copulate, despite repeated exposure to receptive females. Novel environments are stressful to male rats, and induce the activation of endogenous opioids. Treatment with the opioid antagonist naloxone can reverse this novelty-induced stress effect.

Another way to study hypoactive desire in male rats is to examine their sexual responsiveness following multiple ejaculations, a phenomenon known as "sexual exhaustion". Male rats are able to ejaculate several times before becoming unresponsive. During this period of sexual activity, there is a progressive increase in the post-ejaculatory refractory period consonant, with a decrease in the number of intromissions before each ejaculation and a lengthening of the interintromission interval (a state that suggests a progressive loss of erectile function as the number of ejaculations increases). After males become sexually exhausted, they remain unresponsive to female solicitations for up to 72 hrs. Rodriguez-Manzo and colleagues have examined the ability of several classes of drug to increase the responsiveness of these males, including the opioid antagonist naloxone, 8-OH-DPAT, and the α_2 presynaptic autoreceptor antagonist yohimbine. Only yohimbine increased the proportion of males that mounted, intromitted, and displayed an ejaculatory-like behavioral pattern (but without seminal emission), suggesting that a decline in adrenergic activity is an important component of inhibited sexual desire in males.

La Peyronie's disease

An experimental model of transforming growth factor beta1 (TGF-β1)-induced La Peyronie's like condition in the rat has been proposed by Tom Lue's group. The fibrosis is induced by a single injection of TGF-β1 in the tunica albuginea of Sprague Dawley rats. Following TGF-β1 injection, a dramatic influx of inflammatory cells is observed in the tunica albuginea, whereas the normal architecture of the tissue remains preserved. At two weeks, the inflammation

decreases and the tunica albuginea structure is disturbed, which leads to fibrosis development both in the tunica albuginea and the adjacent corpus cavernosum of the penis at six weeks. This fibrosis is considered as a La Peyronie's-like condition because it exhibits biochemical and structural features that are similar to the human La Peyronie's disease plaque. Therefore, this animal model appears as a relevant tool to test innovative La Peyronie's disease pharmacologic strategies.

Hypogonadism/agonadism

In male rats, castration or the administration of androgen synthesis inhibitors, like cyproterone acetate, disrupt and ultimately eliminate copulatory behaviors and penile reflexes progressively over time. They also shrink androgen sensitive peripheral tissues (e.g. penis and prostate). Although the degree of disruption depends on the amount of androgen synthesis inhibition that is induced (e.g. moderate following low doses of cyproterone acetate to total disruption following castration), the amount of time it takes to reach an asymptotic level of behavioral or reflexive performance depends on the level of sexual experience male rats have prior to treatment. In each case, subsequent exogenous administration of androgens or estrogens can restore sexual interest and copulatory behavior, with nonaromatizable androgens (such as dihydrotestosterone) restoring peripheral tissues, and aromatizable androgens (e.g. testosterone) restoring behavioural measures. As with hypogonadal men, restoration of copulatory responses in castrated rats requires a threshold dose of aromatizable androgen that can restore plasma levels lower than those found in gonadally intact, functional animals (higher circulating levels are required for additional anabolic effects on muscle). It is striking, however, that appetitive sexual responses are the least affected by castration and can be maintained for months following. This finding echoes observations that treatment of sex offenders with androgen synthesis inhibitors does not reduce certain appetitive patterns of abuse (e.g. fondling), despite the fact that these men do not achieve erection. There are currently no models of age–related hypogonadism, although a decline in the sexual responsiveness of older male rats (>one year) has been reported in a few studies. However, castrated males can be maintained on subthreshold doses of testosterone prior to behavioral tests, or on a threshold dose followed by a progressive lowering of the dose regimen to mimic a more progressive decline, such as might be seen clinically.

Female Sexual Function

Gonadally-intact female rats of reproductive age go into sexual "heat" every four to five days immediately after ovulation. This process is initiated by the sequential actions of estrogen and progesterone (and possibly also androgens) in the brain and periphery, so that sexual behaviour shows an "estrous cycle". Although women (and certain other primate females) can engage in sexual activity throughout their menstrual cycle, there is a peak rise in female-initiated sexual activity around the time of ovulation, suggesting that estrogen–induced neurochemical systems have been conserved throughout mammalian evolution.

Peripheral sexual reflexes (e.g. vaginal blood flow), copulatory behaviour (solicitations, pacing, lordosis), and appetitive conditioned sexual responses (e.g. conditioned arousal) have been examined in female rats (Fig. 1.4), rabbits, and primates such as macaques. The physiologic mechanisms that regulate vaginal blood flow are extremely similar between species, and more is known about the hormonal and neural regulation of the reflexive posture lordosis (the dorsiflexion of the back that denotes sexual "receptivity" in many species) than any other sexual reflex. Recent work has also begun to elucidate the hormonal and neural control of complex behaviors used by females to regulate the initiation and rate of copulation, offering a real potential to utilize female rats as models for human sexual function.

It is important to emphasize that compared to human males, in whom ability to achieve and maintain erections sufficient for sexual activity is also good for self-esteem related to competent sexual performance, in women there is no clear relationship between physiologic performance and sexual desire. The subjective feeling of sexual arousal

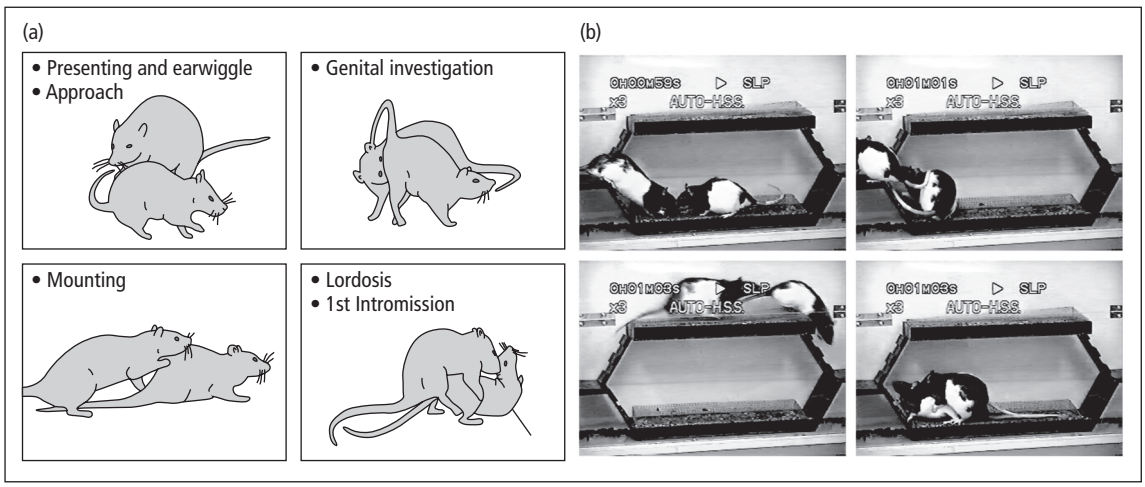

Fig. 1.4 Sexual behaviors displayed by male and female rats. (a) Line drawing of proceptive behaviors (presenting, ear wiggling and approach) and receptive behavior (lordosis) displayed by the female that evokes interest, investigation, chasing, and copulatory responses (mounts, intromissions, and ejaculation) in the male. (b) Female and male sexual behavior in a bilevel testing chamber. Top left: Female (right) makes a headwise orientation toward the male (left) characteristic of a solicitation. Top right: Female hops over the male to reveal her anogenital region, allowing the male to sniff and become aroused. Bottom left: Female runs away, forcing the male to chase her. Bottom right: Female holds a lordosis posture that allows the male to mount and gain vaginal penetration.

results more from cognitive processing of stimulus, meaning and content than from peripheral vasocongestive feedback. Indeed, there are well-identified discrepancies between physiologic and subjective measures of sexual arousal in women, and often no correlation between them (e.g. following treatment with PDE-5 inhibitors). It is not yet known how vaginal responses are integrated with behavioral responses. It remains questionable that increased vaginal blood flow could be perceived by females and participates in the stimulation of behavioral measures of sexual arousal. Accordingly, a more integrative approach is necessary to investigate female sexuality experimentally.

Genital sexual arousal

Upon sexual arousal, the blood supply to the vagina is rapidly increased and at the same time the venous drainage is reduced, thus creating vasocongestion and engorgement with blood. Such an increase in blood flow combined with an enhanced permeability of the capillary tufts induces a neurogenic transudate, which results in vaginal lubrication. From arousal to orgasm, there is also an increase of vaginal luminal pressure.

Reliable and standardized models to study the physiology/pharmacology of female vaginal sexual arousal have been described in dogs, rabbits and rats. In these models, vaginal sexual arousal along with clitoral tumescence is induced by peripheral electrical neural stimulation, while direct measurements of various vaginal physiologic variables are performed. These models have been useful to initiate the exploration of the peripheral physiology of female genital sexual response as well as the consequences of various experimental pathophysiologic conditions (e.g. atherosclerosis or hormonal deprivation).

Female orgasm, the urethra–genital reflex, and the consequences of paced copulation

It is not known whether female rats experience anything like orgasm during sex. However, like males, they display a genital reflex, called the "urethro–genital reflex" that is reminiscent of the

orgasmic response in women. This reflex can be induced and studied in isolation by applying a mechanical stimulation to the urethra of anesthetized, spinalized female rats. The urethro–genital reflex includes rhythmic contractions of the vagina, uterus, and the anal sphincter, as well as the striated pelvic musculature. Stimulation of the urethra may mimic stimulation of the anterior wall of the vagina, which is the area with the highest "erotic" sensitivity. Indeed, the anterior wall of the vagina has a denser innervation than the posterior wall, and the distal area has more nerve fibers than the proximal.

During copulation, female rats typically control the initiation and rate of contact with males. In the same way that ejaculation is critical for conditioned sexual responses in males, the ability of female rats to pace their copulatory contact is critical for the induction of conditioned place or partner preferences. As in males, these consequences of sexual stimulation are blocked by administration of the opioid antagonist naloxone, suggesting that opioid activation is a critical feature of the sexual reward experienced during paced copulation. In addition, pacing imposes a delay between successive vaginal intromissions by the male. This delay distributes the vaginocervical stimulation that females receive over time, an effect that enhances reproductive capability and fertility in the female. Vaginocervical stimulation during orgasm in women may have a similar enhancing effect on reproductive capability.

Copulatory behavior and measures of female sexual motivation or "desire"

As in males, female sexual behavior can be divided into sequential appetitive and consummatory components (Fig. 1.1). In the female rat, these aspects of sexual behavior are also referred to as proceptive and receptive components, two different aspects of sexual behavior displayed by estrous females in the presence of sexually-active males. To solicit attention and approach of the male, the female displays a variety of active proceptive behavioral patterns, including solicitations, hops and darts, and ear-wiggles. As mentioned above, receptivity has been used to describe the behavioral postures assumed by females to allow mounting by a male, with the lordosis reflex being the best known and most studied response. Unfortunately, there is no counterpart for lordosis in women. In contrast, psychologic arousal or desire in women is likely to be very close to proceptivity. For this reason, the study of proceptive behaviors is also relevant to any preclinical investigation of potential of compounds for the treatment of female sexual disorders or dysfunctions (FSD). Indeed, recently it has been reported that the melanocortin agonist, PT-141, increased rates of sexual solicitation and hops and darts in female rats selectively. This same drug increased female-initiated sexual activity in early Phase IIa clinical trials. These two observations were critically important to begin to establish solicitation as a valid model of female sexual desire.

Models of female sexual dysfunctions

Hypogonadism/agonadism and hypoactive sexual desire

Although there are currently no established models of female sexual dysfunction in rats, there are several reasons to believe that such models could exist. The most obvious would be the consequences of hypogonadism induced by ovariectomy followed by maintenance with different doses of estrogen alone, or estrogen and progesterone. Ovariectomized rats treated with estrogen and progesterone display a complete pattern of proceptive and receptive behaviors, whereas those treated with estrogen alone display no proceptive behaviors, low levels of lordosis, and high rates of rejection responses. Certain pharmacologic treatments (e.g. apomorphine, oxytocin, PT-141), are able to increase proceptive behaviors and reduce rejection responses in ovariectomized females treated or maintained on estrogen alone. This pattern of data suggests that such drugs may be useful in the treatment of hypoactive sexual desire disorder, with or without accompanying hypogonadism.

A similar loss of interest in sexual activity occurs during the phenomenon of "estrous termination". Estrous termination occurs progressively after the female receives a requisite number of intromissions and ejaculations during sex. It can also be induced by

manual vaginocervical stimulation using a lubricated glass rod that approximates the size of a male rat penis, and that is inserted to mimic the stimulation received during intromission. The first behavioral set to disappear is solicitation, and this precedes a rise in rejection responses. Females given vaginocervical stimulation also show a faster loss of lordosis over the next 12 hrs, compared to females given sham stimulation. It would appear, then, that sexual stimulation in females, as in males, activates inhibitory systems that bring about refractoriness. It is not yet known how different pharmacologic treatments might delay the onset of estrous termination, and whether such effects might prove useful in treatment of low desire.

It will be important to consider how androgen administration may alter sexual behavior in ovariectomized females, with or without estrogen treatment. Currently, combined androgen—estrogen treatment is used in postmenopausal women to restore sexual desire and arousal. It should be straightforward to examine this combined hormone therapy in ovariectomized rats and categorize the effects, along with studying potential mechanisms (e.g. steroid receptor activation and interaction, role of peripheral sex hormone binding globulins).

It will also be critical to study the sexual behavior of older female rats. Female rats have a homologue of "menopause" in which ovarian function is disrupted then declines to a continuous state of vaginal diestrus (accompanied by a progressive atrophy of the vagina and clitoris). It is not known how these females would respond to sexual advances by a male, or to manually applied vaginocervical stimulation.

Hypoactive sexual arousal

Treatment of rats with the peripheral nitric oxide inhibitor nitro-L-arginine-methyl ester (L-NAME) reduces vaginal blood flow. It is not yet known if this treatment alters female sexual behavior.

Hypoactive sexual desire/conditioned inhibition

Female rats treated with the opioid antagonist naloxone during paced copulatory trials do not form conditioned place or partner preferences. Such preferences are typically examined on a final test, when the drug is not administered (thus revealing the necessity of opioid reward during paced copulation). However, during this final test without the drug, females previously treated with naloxone display a conditioned disruption of solicitation and lordosis relative to saline-treated females, despite being primed fully with estrogen and progesterone. As a result, males engage in fewer intromissions and achieve fewer ejaculations with those females. A pattern of diminished sexual solicitation and receptivity, which leads to more restricted sexual contact with males, is analogous in many ways to the pattern of sexual behavior displayed by women with hypoactive sexual desire disorder. It is not yet known if this pattern of disrupted appetitive sexual behavior can be restored by pharmacologic or experiential treatments that increase desire in women.

General Conclusion

Real progress has been made in understanding the neuroanatomical and neurochemical mechanisms of erection, ejaculation, solicitation, and other sexual responses, and in the design of rational pharmacologic treatments for certain sexual dysfunctions. We have begun to examine the mechanisms that underlie desire, and how sexual stimulation and reward impact on endpoints like sexual arousal, desire, attractiveness, and even mate choice. Progress in these areas could not have been made without the help of animal models. The evolution of sexual physiology and behaviour have been highly conserved, therefore animal models of human sexual response can be used successfully as preclinical tools so long as the functional endpoints are homologous or analogous, and carry predictive validity. When setting up testing paradigms to study preclinical models of human sexual function in laboratory animals, it is essential to ask the animals human questions that they can answer in their own species–specific manner. Although standardization of models (and of paradigms between laboratories) is critical, this should never limit the inspired intuition of researchers or clinicians to envision new models or paradigms.

Guidelines and Recommendations for Setting up a Preclinical Lab in Sexual Medicine

Research questions

The selection of a research topic is the most fundamental decision in establishing a preclinical laboratory in sexual medicine. This decision will control all subsequent considerations, such as research models and techniques, collaborative arrangements and funding needs. Clearly, the choice of a research program will be heavily influenced by one's interests and background. However, an often overlooked, but important, consideration is opportunity, such as the opportunity to achieve distinction, funding opportunities, or the opportunity to establish collaborative relationships with research groups that may have research techniques that can be usefully applied to questions in sexual medicine.

For the starting investigator, an important question to ask is what will be necessary to achieve both success and distinction? This can be difficult in an area which is already somewhat mature or has a number of well-established labs working in it. In a mature field, many of the bigger questions may have been answered. This may leave only opportunities to investigate details of previously-identified mechanisms, with reduced opportunities for recognition. Trying to compete against established labs can also lead to difficulty in achieving recognition. The new investigator with a small laboratory still developing techniques will be hard pressed to publish the first or the most comprehensive results compared to a larger established lab.

One approach is to identify research areas that are comparatively understudied. Some of the more fundamental questions may remain to be answered, with correspondingly higher recognition. The disadvantage to this approach is that understudied areas may lack well defined research models or they may be understudied precisely because they are too challenging. However, new investigators can often find a niche because they can see research fields with fresh eyes.

Selection of models

The selection of the appropriate model is crucial to progress. This chapter and others referenced here provide useful considerations on this topic. The new investigator should also seek advice from mentors and investigators in sexual medicine to help make these decisions about experimental models. Luckily, the field of sexual medicine is fairly new, with a relatively small, collegial community. Established investigators want to encourage growth of the field, and therefore are generally willing to help new investigators.

Experimental techniques

The choice of research models will dictate what research techniques will be needed. If these are new methods for the investigator, there are many approaches to acquiring the necessary technical skills. Some may be relatively straightforward to learn on one's own, using the literature. However, it may save considerable time to visit labs using the techniques to learn the little details and learn to troubleshoot problems. Other techniques may require more formal training. Many courses on scientific techniques are offered by professional societies and equipment/reagent manufacturers. Longer visits or mini-sabbaticals to other labs may also provide the necessary training.

Collaborations

Developing collaborative relationships can be extremely useful, or even essential for the new investigator, especially someone with a demanding clinical practice. Except in very large institutions, there are unlikely to be many other researchers in sexual medicine. Therefore, the most likely collaborators will be in related areas of cellular and molecular biology, physiology and pharmacology, endocrinology, or neuroscience, depending on the research topic. The collaborative arrangements can range from simply trading technical help to a complete sharing of labs. Typically, these relationships will begin with rather limited interactions and grow over time. It is probably unrealistic to expect that a potential collaborator will commit to an extensive interaction from the start. It is also likely that attempts at collaboration are not successful. Beyond the scientific interaction, the personal relationship is important.

In a successful collaboration, everyone must feel that they are benefiting. People often will be willing to provide limited help (such as teaching a

technique) without expecting something in return. For a longer-term project, they will often need reasons to justify their expenditure of time and effort. The new investigator must identify the possible benefits of collaboration. These could be an entry into a young, interesting field with many opportunities, access to new sources of funding, or additional people, such as fellows, to work in their lab.

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