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REVIEW

Advanced genetic and viral methods for labelling and manipulation of oxytocin and vasopressin neurones in rats

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Abstract

Rats have been widely used as one of the most common laboratory animals for biological research, because their physiology, pathology, and behavioral characteristics are highly similar to humans. Recent developments in rat genetic modification techniques have now led to further their utility for a broad range of research questions, including the ability to specifically label individual neurones, and even manipulate neuronal function in rats. We have succeeded in generating several transgenic rat lines that enable visualization of specific neurones due to their expression of fluorescently-tagged oxytocin, vasopressin, and c-*fos* protein. Furthermore, we have been able to generate novel transgenic rat lines in which we can activate vasopressin neurones using optogenetic and chemogenetic techniques. In this review, we will summarize the techniques of genetic modification for labeling and manipulating the specific neurones. Successful examples of generating transgenic rat lines in our lab and usefulness of these rats will also be introduced. These transgenic rat lines enable the interrogation of neuronal function and physiology in a way that was not possible in the past, providing novel insights into neuronal mechanisms both in vivo and ex vivo.

Keywords Transgenic rat · Oxytocin · Arginine vasopressin · Optogenetics · DREADDs

Introduction

Reliable and efficient methods of gene transfer are desired to successfully establish genetically modified animal models, and to determine how genotype affects phenotype. The first genetically modified animal was generated by Jaenisch et al. in 1974 (Jaenisch and Mintz 1974). They inserted a DNA virus into an early-stage mouse embryo and the inserted genes were present in every cell; however, they were not inherited by the offspring. Gordon et al. (Gordon and Ruddle 1981), Costantini et al. (Costantini and Lacy 1981), and Brinster et al. (1981) were the first to succeed in transmission of the modified genome to the subsequent generation by injecting purified DNA into single-cell mouse embryo. Classically, the transgenic method involves insertion of a foreign gene at a random position in the genome. In the past decades, however, several novel genome editing technologies have enabled us to now potentially manipulate any gene in many different cell types and organisms, typified by zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas-based RNA-guided DNA endonucleases (Meek et al. 2017).

Arginine vasopressin (AVP) and oxytocin (OXT) are synthesized in the magnocellular neurosecretory cells (MNCs) of the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus. They project axon terminals to the posterior pituitary and secrete AVP and OXT into the systemic circulation with action potential-dependent regulation (Brownstein et al. 1980). Plasma AVP acts on the kidney as an antidiuretic hormone via the V2 receptor (Holmes and Russell 2004), while plasma OXT regulates well-characterized processes in uterine contraction and lactation (Marshall 2011). Somato-dendritic release of AVP and OXT from the MNCs can also mediate various central actions (de Kock et al. 2003; Ludwig and Leng 2006).

In the past, it was difficult to identify or visualize neurones unless they were fixed and stained using immunohistochemistry or in situ hybridization. The ability to identify specific neurones without the need for antibodies or probes signifies a major methodological refinement, making the experiment much easier as well as providing deeper understanding. For

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example, it is extremely useful when we conduct electrophysiology from "live" neurones, which can be readily identified in a rat line with fluorescent-tagged neurones (Ohbuchi and Ueta 2014; Ohkubo et al. 2014a, b). In addition, rapid development of chemogenetics and optogenetics has more recently enabled us to better understand the relationship between central nervous activity and diverse behaviors (Deisseroth 2011; Magistretti and Allaman 2013). Importantly, we can now insert genetically modified channels or receptors into specifically targeted neurones. This ensures that only the specific neuronal type can be manipulated by the experimental stimuli of specific wavelength light in optogenetics or specific drug in chemogenetics.

Our group has established several transgenic rat lines to date. In this review, we will summarize the brief description of the methods of gene transfer into the central nervous system. We will also present examples of the transgenic rat lines in our lab, including novel technologies of optogenetics and designer receptors exclusively activated by designer drugs (DREADDs).

Methods of the gene transfer

There are various methods for gene transfection. The ideal method of gene transfer depends on the cell type and purpose for which researchers want to conduct. They should have high transfection efficiency, low cell toxicity, minimal effects on normal physiology, and be easy to use and reproducible (Kim and Eberwine 2010). Here, we outline representative examples of the main methods used for gene transfer into the central nervous system, and briefly address their advantages and disadvantages. Summary figure of the methods of the gene transfer into central nervous system is also presented (Fig. 1).

In vivo viral transfection directly into the specific brain area

Virus-mediated transfection, also known as transduction, is one of the most commonly used molecular intervention methods in clinical and experimental research (Pfeifer and Verma 2001; Bostick et al. 2007). It is highly efficient and easy to achieve sustainable transgene expression in dissociated neurones, slices, and in vivo (Kim and Eberwine 2010). Local administration of viral vectors containing the foreign gene is introduced into neurones by exploiting the cell entry system originally possessed by the virus. Each viral vector infects into neurones via a unique receptor present on the neurones surface. Adeno-associated virus (AAV), adenovirus, retrovirus, and lentivirus are commonly used for the transduction (Technologies 2012). Besides its utility, there are some drawbacks, including immunogenicity and cytotoxicity. As viral vectors integrate into the host genome randomly, they can result in an insertional mutation. Mutations of this type are particularly problematic when they cause disruption of tumor suppressor genes, activation of oncogenes, or interruption of essential genes (Woods et al. 2003). There are also potentially hazards to laboratory personnel to consider when working with viruses. Another notable restriction is that this method can often be inappropriate for inserting larger-sized genes (Hacein-bey-abina 2002; Woods et al. 2003; Kim and Eberwine 2010). The method is also highly dependent on technical proficiency, due to the degree of difficulty in microinjecting accurately into discrete nuclei within targeted regions of a rodent brain. In addition, variability in the infectivity of the viral vector preparations make it difficult to achieve consistent expression levels of foreign genes (Vorburger and Hunt 2002; Kim and Eberwine 2010).

Viral-mediated transfection has still been widely used despite these disadvantages because high transfection efficiency and sustainable expression can be easily achieved, thus advantages surpass disadvantages. Many excellent studies have been published by using this method on AVP and OXT neurones (Fields et al. 2012; Knobloch et al. 2012; Ponzio et al. 2012; Eliava et al. 2016; Grund et al. 2017; Menon et al. 2018).

Transgenic approach

Insertion of foreign genes at random positions in the genome

When DNA containing a specific gene is injected into a germ cell or a fertilized egg, the DNA is inserted at a random position in the genome and will be inherited to the next generation. Gene expression can be restricted to specific tissues or neurones by engineering the relevant promoter, an enhancer, an intron, and a polyadenylation signal. However, the expression level of the transgene will mainly depend on the position of its insertion in the genome and the copy number. In case of rats, pronuclear microinjection of fertilized eggs has been used to generate transgenic rats, mainly for gain-of-function studies (von Horsten et al. 2003; Popova et al. 2005; Leon et al. 2010). Usually, foreign genes are inserted in one place in the genome with multiple copies forming a concatemer. Pronuclear microinjection is widely used; however, the insertion efficiency of the foreign gene is often poor. Several hundred fertilized eggs are normally required. In addition, since the cytoplasm of fertilized eggs is opaque, accurate pronuclear injection is sometimes difficult and efficiency is further reduced.

Recently, a method using lentivirus, which is a kind of retrovirus, has attracted attention (Cockrell and Kafri 2007; Pfeifer et al. 2010). Notably, lentivirus can easily infect non-dividing cells. Foreign genes delivered by lentiviral transduction are also less susceptible to silencing by host cells.

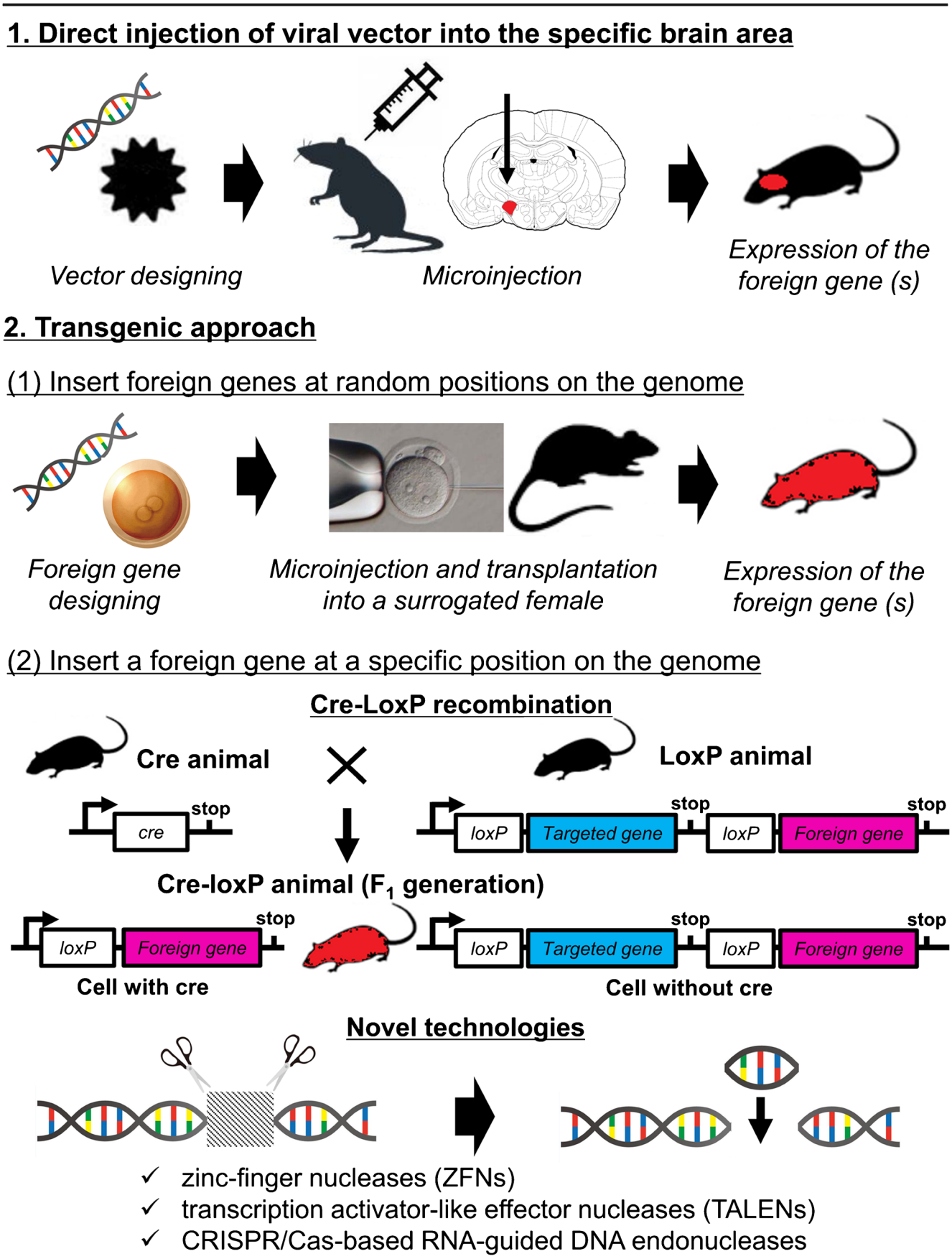
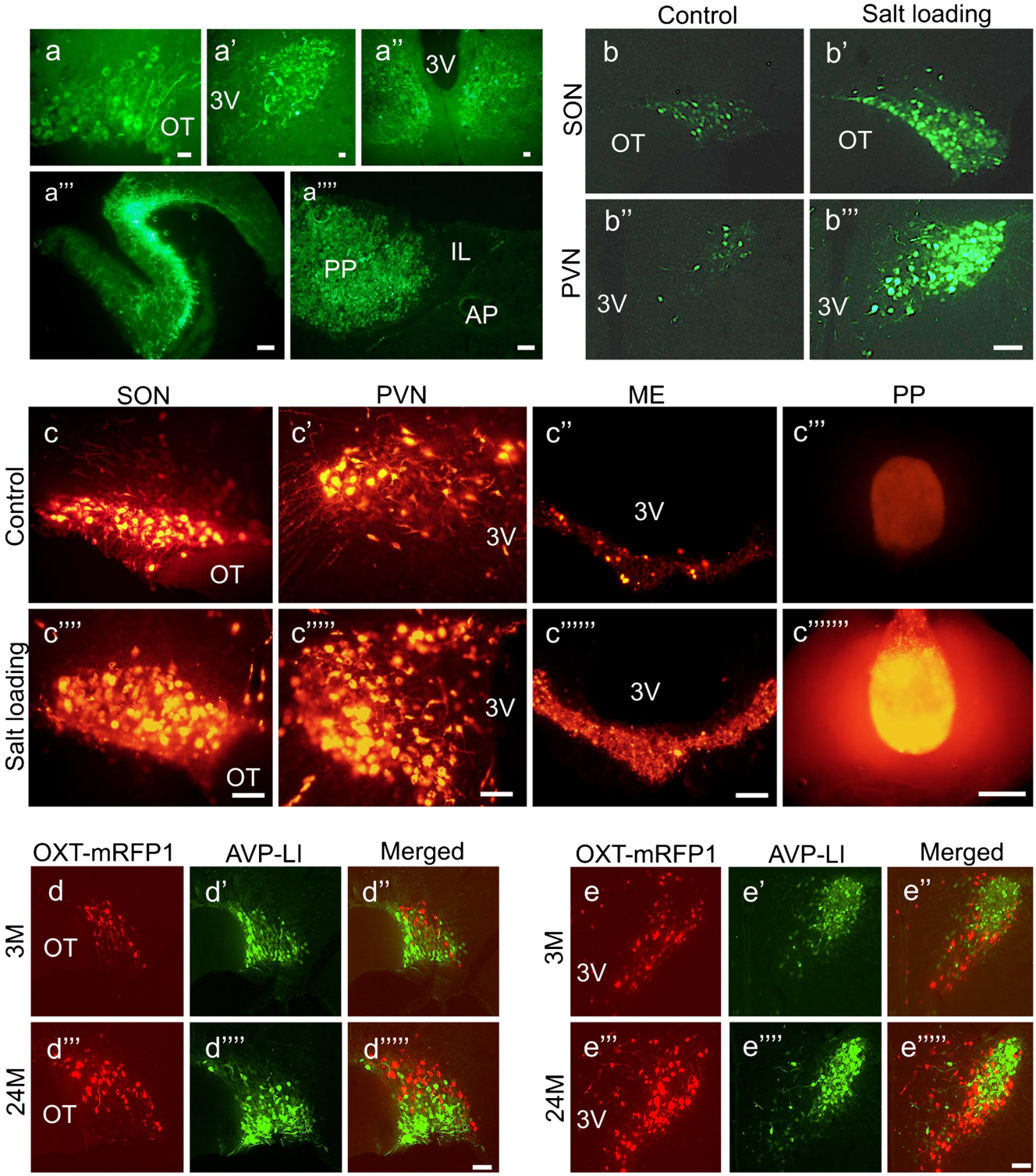


Fig. 1 Representative methods of the gene transfer into the central nervous system. Methods of gene transfer into the central nervous system are described



Lentivirus can be microinjected into the perivitelline space which is outside of the egg cell, resulting in much higher efficiency of gene transfer than pronuclear microinjection.

Another possible candidate for gene transfer is the transposon method (Burns and Boeke 2012; Hemantha 2014). The transposon method combines retroviral DNA integration with DNA cleavage events involved in immunoglobulin gene formation. Therefore, the foreign gene is inserted using the same mechanisms that allow transposons to be inserted into the genome. To date, various transposons suitable for gene

◀ **Fig. 2** Fluorescent visualization of AVP and OXT neurones. Endogenous fluorescence of enhanced green fluorescent protein (eGFP) in the supraoptic nucleus (SON) (**a**), paraventricular nucleus (PVN) (**a'**), suprachiasmatic nucleus (SCN) (**a"**), infundibulum (**a"'**), and posterior pituitary (PP) (**a"'**) in an AVP-eGFP transgenic rat line. Scale bars, 50 μm. *OT* optic tract, *3V* third ventricle, *IL* intermediate lobe, *AP* anterior pituitary. AVP-eGFP was upregulated after chronic salt loading in the SON (**b'**) and PVN (**b"'**) compared to control (**b**, **b"**) in an AVP-eGFP transgenic rat line. Scale bars, 50 μm. *OT* optic tract, *3V* third ventricle. Endogenous florescence of monomeric red fluorescent protein 1 (mRFP1) in the SON (c), PVN (**c'**), median eminence (ME) (**c"**), and PP (**c"'**) in an OXT-mRFP1 transgenic rat line. Chronic salt loading increased OXT-mRFP1 (**c"'**–**c"""'**) compared to control (**c**–**c"'**). Scale bars, 100 μm (**c"'**–**c"""**) and 1 mm (**c"""'**). *OT* optic tract, *3V* third ventricle. Endogenous mRFP1 (**d**, **d"'**, **e**, **e"'**) with AVP-like-immunoreactivity (LI) (**d'**, **d"'**, **e'**, **e"'**) in the SON (**d**–**d"'**) and PVN (**e**–**e"'**) obtained from 3-month-old (**d**–**d"**, **e**–**e"**) and 24-month-old (**d"'**–**d"'**, **e"'**–**e"'**) OXT-mRFP1 transgenic rat line. Merged images are displayed (**d"**, **d"'**, **e"**, **e"'**). Scale bars, 50 μm. *OT* optic tract, *3V* third ventricle. Reproduced modification from ref. Ueta et al. (2005) Transgenic expression of enhanced green fluorescent protein enables direct visualization for physiological studies of vasopressin neurons and isolated nerve terminals of the rat. *Endocrinology* 146:406–413, Fig. No. 2; ref. Katoh et al. (2011) Highly visible expression of an oxytocin-monomeric red fluorescent protein 1 fusion gene in the hypothalamus and posterior pituitary of transgenic rats. *Endocrinology* 152:2768–2774, Fig. No. 4 with permission of the Endocrine Society; and ref. Ohno et al. (2017) Increased oxytocin-monomeric red fluorescent protein 1 fluorescent intensity with urocortin-like immunoreactivity in the hypothalamo-neurohypophysial system of aged transgenic rats. *Neurosci. Res.*, Fig. No. 6 with the permission of Elsevier

transfer in vertebrates and invertebrates have been identified (Hamlet et al. 2006; Sasakura et al. 2007; Ivics et al. 2009).

Insertion of a foreign gene at a specific position on the genome

Insertion of a foreign gene into a specific position on the genome is called gene targeting (Kolb and Siddell 1996; Persic et al. 1997; Shashikant et al. 1998). Strictly, animals created by gene targeting are defined as transgenic animals. However, they are often distinguished from those created by inserting foreign genes at a random position, because homologous recombination is used for the recombination (Agarwal et al. 2007; Trudeau et al. 2013). Using this method, an endogenous gene can be destroyed by replacing an essential part of the gene with a foreign gene, this enabling us to generate "knock-out" as well as "knock-in" animals. Furthermore, this type of homologous recombination can generate proteins that have altered thermo- and mechanical stability, enzyme substrate specificity, and optogenetic properties (Trudeau et al. 2013). However, the species for which targeted gene recombination techniques have been established is very limited. LoxP DNA sequences, recognized by the DNA recombinase "Cre," are often inserted flanking specific genes (Kolb and Siddell 1996; Akagi et al. 1997; Nagy 2000). This technique can also be used for generating conditional knockout animals in which a specific gene is disrupted in a specific cell population and at a specific timing.

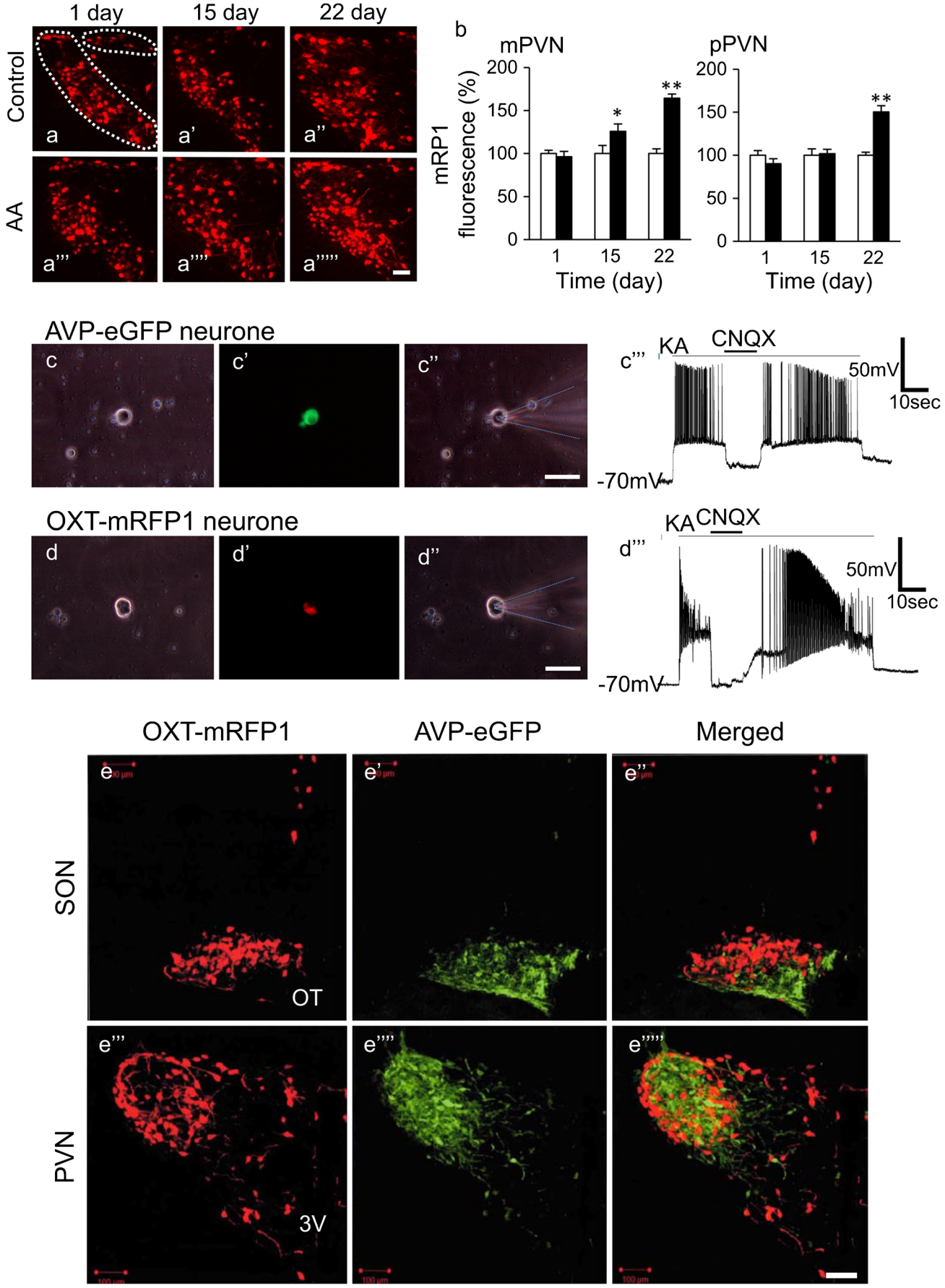
The use of gene targeting has been somewhat limited due to difficulties associated with low insertion rate of foreign genes. However, the recent development of novel gene targeting techniques, including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas-based RNA-guided DNA endonucleases, has now allowed us to generate genetically modified animals with greater efficiency and confidence (Urnov et al. 2005; Christian et al. 2010; Morbitzer et al. 2010; Gasiunas et al. 2012; Jinek et al. 2012). These techniques utilize engineered nucleases composed of sequence-specific DNA-binding domains fused to a non-specific DNA cleavage module (Meek et al. 2017). With these types of advances, it is expected that gene recombination in various animal species will be simplified and more available in the future.

Examples of transgenic rat lines in our lab

Fluorescent visualization of AVP and OXT neurones

Electrophysiological studies have characterized the electrical properties of AVP and OXT in the SON and PVN of the hypothalamus both in in vivo and in ex vivo brain preparations (Yamashita et al. 2002). The patch-clamp methodology has been used for single-cell dye labeling in cultured neurones, brain slices, and in vivo preparations with fluorescent probes via patch pipettes. While individual neurones can be identified by dye uptake during in vivo experiments, then confirmed using immunohistochemistry with specific antibodies, it is more difficult to distinguish between different types of "live" neurones in ex vivo brain preparations (Armstrong et al. 1994). Therefore, the ability to easily identify these neurones or nerve terminals would be greatly beneficial during physiological characterization of AVP and OXT.

In 2005, we designed a fusion gene of AVP and enhanced green fluorescent protein (eGFP) (Tsien 1998) by using pRVP[S-E/BD-S]Not (Zeng et al. 1994), and generated a transgenic rat line (Ueta et al. 2005) (Fig. 2a–a"'). The AVP-eGFP transgenic rats maintain normal body fluid homeostasis and intact humoral responses of both AVP and OXT (Fujio et al. 2006). Interestingly, AVP-eGFP fusion protein expression fluctuates in response to chronic salt loading, bilateral adrenalectomy, and nociceptive stimulation (Fujio et al. 2006; Shibata et al. 2007; Suzuki et al. 2009) (Fig. 2b–b"'). It was also upregulated in kainic acid-induced seizure (Iwanaga et al. 2011; Ohno et al. 2012). In particular, the AVP-eGFP transgenic rat line has been extremely useful for the characterization of AVP neurones in


1 day
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◀ **Fig. 3** Functional studies of AVP-eGFP and OXT-mRFP neurones. OXT-mRFP1 in the PVN (**a**–**a**”“’) after chronic nociceptive model obtained from an OXT-mRFP1 transgenic rat line. Sections were obtained at days 1 (**a**, **a**”’), 15 (**a’**, **a**”“), and 22 (**a**”, **a**”“’) in control (**a**–**a**”) and adjuvant arthritis (AA) model (**a**”’–**a**”“’) rats. Fluorescent signals from the magnocellular (m) and parvocellular divisions (p) of the PVN were measured separately. The mPVN and pPVN are surrounded with a white dotted line. Scale bar, 100 μm. The average mRFP1 intensities are shown for mPVN and pPVN (**b**). Values are presented as means ± SEM (n=6–8 per group at each point). *$P < 0.05$, **$P < 0.01$ compared with each control. Whole-cell patch-clamp recordings from magnocellular neurosecretory cells (MNCs) isolated from the SON obtained from AVP-eGFP (**c**–**c**”’) and OXT-mRFP1 transgenic rat lines (**d**–**d**”’). The depolarization induced by kainic acid (KA) (100 μM) could trigger a different pattern of action potentials in AVP neurone (**c**”’) and OXT neurone (**d**”’). The depolarization and action potentials were highly attenuated by CNQX [6-cyano-7-nitroquinoxaline-2,3(1H4H)-dione] (10 μM). Scale bars, 50 μm. Endogenous mRFP1 (**e**, **e**”’) and eGFP (**e’**, **e**”“) in the SON (**e**–**e**”) and PVN (**e**”’–**e**”“’) could be observed in an AVP-eGFP and OXT-mRFP1 double transgenic rat. Merged images are also represented (**e**”, **e**”“’). Scale bar, 100 μm. *OT* optic tract, *3V* third ventricle. Reproduced modification from ref. Matsuura et al. (2015): Fluorescent visualization of oxytocin in the hypothalamo-neurohypophysial/-spinal pathways after chronic inflammation in oxytocin-monomeric red fluorescent protein 1 transgenic rats. *J Neuroendocrinol* 27: 636–646, Fig. No. 3 and 4; ref. Ohkubo et al. (2014b) Electrophysiological effects of kainic acid on vasopressin-enhanced green fluorescent protein and oxytocin-monomeric red fluorescent protein 1 neurones isolated from the supraoptic nucleus in transgenic rats. *J Neuroendocrinol* 26, Fig. No. 1 with permission of John Wiley and Sons; and ref. Katoh et al. (2011) Highly visible expression of an oxytocin-monomeric red fluorescent protein 1 fusion gene in the hypothalamus and posterior pituitary of transgenic rats. *Endocrinology* 152:2768–2774, Fig. 4 with permission of the Endocrine Society

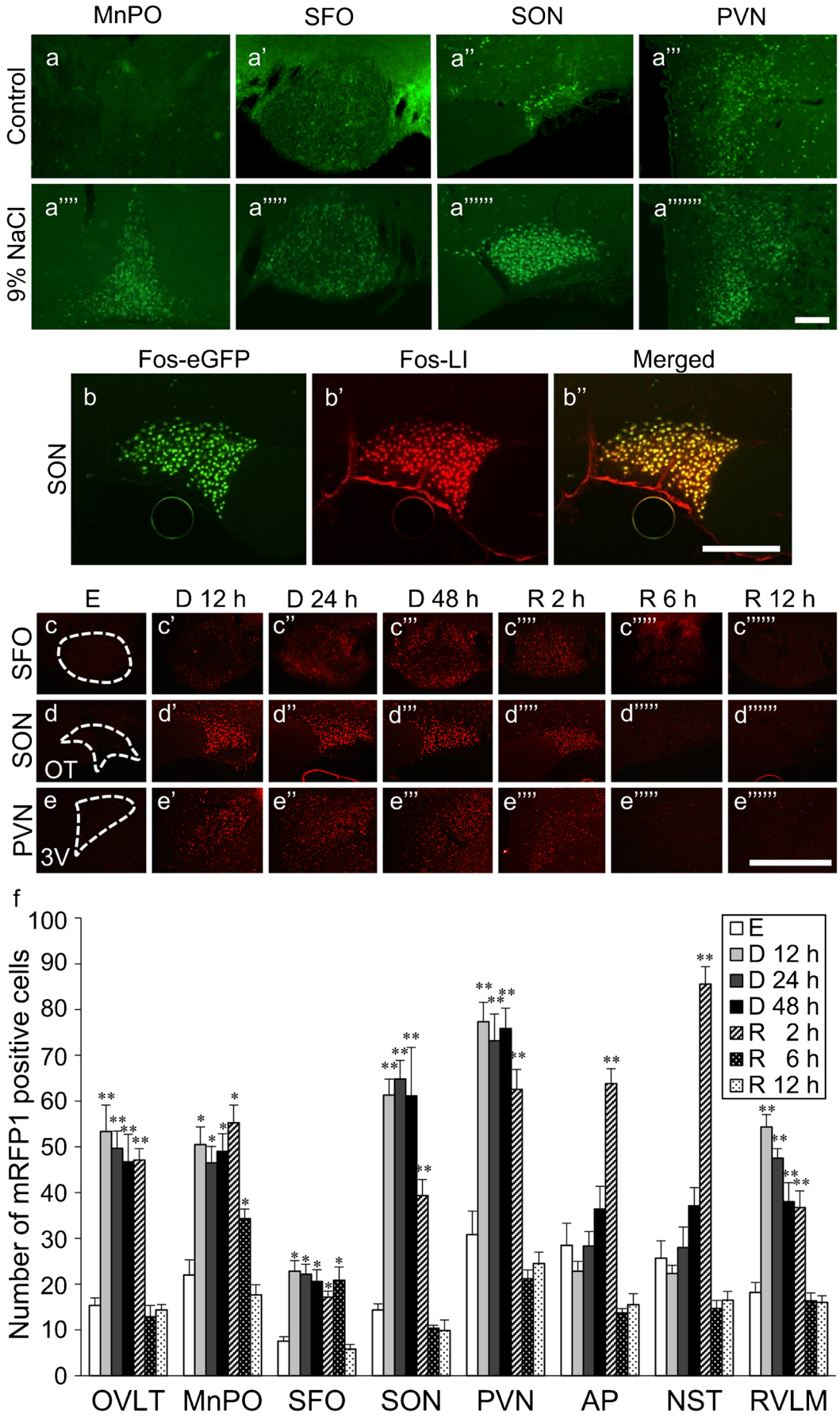
electrophysiology (Ohbuchi et al. 2009, 2010a, b; Ohkubo et al. 2014a, b) and in calcium imaging (Kortus et al. 2016). Recently, a novel system using an optical fiber has enabled us to monitor the intensity of eGFP signals from within the brains of living AVP-eGFP transgenic rats (Iijima et al. 2017). AVP-eGFP can be observed in extra-hypothalamic regions as well. We observed AVP-eGFP in the piriform cortex, reticular thalamic nucleus, medial amygdala, Edinger-Westphal nucleus, and median raphe nucleus after colchicine i.p. injection (unpublished data). Other groups have revealed that AVP-eGFP was also detectable in the olfactory bulb (Tobin et al. 2010) and retinal ganglions (Tsuji et al. 2017) by using this transgenic rat line.

A transgenic rat line that expresses monomeric red fluorescent protein 1 (mRFP1) fused to OXT was created in 2011 (Katoh et al. 2011) (Fig. 2c–c”“”“’) by microinjection of a chimeric OXT-mRFP1 bacterial artificial chromosome (BAC) construct into fertilized eggs. The OXT-mRFP1 appears to be regulated similarly to the endogenous OXT gene. Intraperitoneally administered cholecystokinin (CCK)-8, which selectively activates oxytocin (OXT) neurones in the hypothalamus, also significantly increased OXT-mRFP1 intensity (Katoh et al. 2014; Motojima et al. 2016). Consistent with endogenous OXT, OXT-mRFP1 signal was also upregulated as the rats grow older (Ohno et al. 2017) (Fig. 2d–d”“’, e–e”“’). Since oxytocin is known to be involved in various functions including sensory modulation and autonomic nervous function, the OXT-mRFP1 transgenic rat line has already proved very useful for observing altered OXT synthesis in many experimental conditions. Matsuura et al. have reported that mRFP1 fluorescence was significantly increased in the hypothalamus after acute and chronic nociceptive stimuli (Matsuura et al. 2015, 2016) (Fig. 3a–a”“’, b). Interestingly, they could even observe mRFP1 granules in axon terminals in the spinal cord, due to OXT neuronal projections from the parvocellular neurones of the PVN (Matsuura et al. 2015). OXT-mRFP1 was also increased in cisplatin-induced anorexia (Arase et al. 2017), which is unsurprising because OXT is recognized as one of the anorexic hormones. As with the AVP-eGFP transgenic rat line, this transgenic rat line is a very powerful tool for investigating the characteristics of OXT neurones in electrophysiology (Ohkubo et al. 2014a, b) and calcium imaging (Kortus et al. 2016) (Fig. 3c–c”’, d–d”’). We should note that, both in AVP-eGFP and OXT-mRP1 transgenic rat lines, upregulation of fluorescent protein intensity does not always mean the upregulation of AVP or OXT synthesis. Because it could be possible that transportation or release of AVP or OXT is obstructed and we may just be observing the fluorescent signal from accumulated AVP or OXT.

Double transgenic rats, which carry both AVP-eGFP and OXT-mRFP1 fusion genes, can be generated by crossing an AVP-eGFP transgenic rat and an OXT-mRFP1 transgenic rat (Katoh et al. 2011) (Fig. 3e–e”“’). These transgenic rat lines could be utilized in the study of the physiological role of AVP and OXT neurones in the central nervous system. Further, the dynamics of AVP and OXT synthesis and secretion in living neurones, either in dispersed culture, organotypic culture, or living slices or, indeed, by using advanced imaging techniques in the intact organism could be assessed. In addition, in vivo fiber-optic probes could be used to monitor fluorescent protein levels in the hypothalamus and in the pituitary and, possibly, coupled with in vivo electrophysiological recordings.

Fluorescent visualization of neuronal activity

Induction of immediate early genes (IEGs) has been used to map neuronal activity in the brain (Armario 2006; Terleph and Tremere 2006), as they are activated transiently and rapidly in response to a variety of cellular stimuli. Many IEG products are naturally transcription factors or other DNA-binding proteins; however, other important classes of IEG products have been proposed as secreted proteins, cytoskeletal proteins, and receptor subunits. About 40 cellular IEGs have been identified to date (de Bartolomeis et al. 2017).


MnPO
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Number of mRFP1 positive cells
OVLT
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AP
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◀ **Fig. 4** Fluorescent visualization of neuronal activity. Expression of nuclear c-*fos*-eGFP in the median preoptic nucleus (MnPO) (**a**, **a**”“), subfornical organ (SFO) (**a’**, **a**”“’), supraoptic nucleus (SON) (**a**”, **a**”““), paraventricular nucleus (PVN) (**a**”’, **a**”““’) 90 min after intraperitoneal administration of 0.9% saline (**a**–**a**”’), 9% hypertonic saline (**a**”“–**a**”““’) in a c-*fos*-eGFP transgenic rat line. Scale bar 100 μm. Endogenous c-*fos*-eGFP (**b**) and Fos-like immunoreactivity (LI) (**b’**) was merged (**b**”) in the SON 90 min after acute osmotic (9% saline) stimulation. Scale bar, 50 μm. Examples of c-*fos*-mRFP1 expression patterns in c-*fos*-mRFP1 transgenic rat line. The white dotted lines show the location of analysis (**c**, **d**, **e**) in the SFO (**c**–**c**”““), SON (**d**–**d**”““), and PVN (**e**–**e**”““). *E* ad libitum to water (**c**, **d**, **e**), *D 12 h* water deprivation for 12 h (**c’**, **d’**, **e’**), *D 24 h* water deprivation for 24 h (**c**”, **d**”, **e**”), *D 48 h* water deprivation for 48 h (**c**”’, **d**”’, **e**”’), *R 2 h* water deprivation for 46 h + ad libitum to water for 2 h (**c**”“, **d**”“, **e**”“), *R 6 h* water deprivation for 46 h + ad libitum to water for 6 h (**c**”“’, **d**”“’, **e**”“’), *R 12 h* water deprivation for 46 h + ad libitum to water for 12 h (**c**”““, **d**”““, **e**”““). Scale bar 500 μm. Changes in number of c-*fos*-mRFP1 positive cells after chronic osmotic stimulation in c-*fos*-mRFP1 transgenic rat line (**f**). Values are presented as means ± SEM ($n = 4–12$ each). $*P < 0.05$, $**P < 0.01$ compared to E and R 12 h. Reproduced modification from ref. Katoh et al. (2014) Fluorescent visualisation of the hypothalamic oxytocin neurones activated by cholecystokinin-8 in rats expressing c-fos-enhanced green fluorescent protein and oxytocin-monomeric red fluorescent protein 1 fusion transgenes. *J Neuroendocrinol* 26, Fig. No. 1 and 2 and ref. Yoshimura et al. (2013) A c-fos-Monomeric Red Fluorescent Protein 1 Fusion Transgene is Differentially Expressed in Rat Forebrain and Brainstem after Chronic Dehydration and Rehydration. *J Neuroendocrinol* 25, Fig. No. 2 and 4 with permission of John Wiley and Sons

Among the IEGs, Fos has been used as one of the most popular markers for neuronal activity (Morgan et al. 1987; Herrera and Robertson 1996; Harris 1998; Armario 2006; Shi et al. 2008). We believed that Fos detection could be greatly facilitated by the use of a fluorescent reporter, and thus generated a c-*fos*-eGFP transgenic rat line that carries a fusion gene consisting of the c-*fos* coding sequence in frame with eGFP under the control of c-*fos* gene regulatory sequences (Katoh et al. 2014) (Fig. 4a–a”““’). The c-*fos*-eGFP BAC transgenic construct was purified and microinjected into the perinuclear of the fertilized eggs. c-*fos* eGFP was merged with the endogenous Fos protein (Fig. 4b–b”). The fluorescent-tagged Fos was visible after acute and chronic osmotic challenges (Katoh et al. 2014; Yoshimura et al. 2014). Acute nociceptive stimuli, which was induced by formalin or capsaicin, also induced c-*fos*-eGFP in the hypothalamus and spinal cord (Motojima et al. 2017). The induction of c-*fos*-eGFP following exposure to acute nociceptive stimuli was more sensitive than endogenous Fos, suggesting that this transgenic rat line could be a useful model for the study of nociceptive pathways and processing (Motojima et al. 2017).

We also generated a c-*fos*-mRFP1 transgenic rat line (Fujihara et al. 2009). The purified c-*fos*-mRFP1 BAC transgenic construct was microinjected into the pronuclear of fertilized eggs donated from Wistar rats. Consistent with our findings for c-*fos*-eGFP, we could observe robust induction of c-*fos*-mRFP1 located in the nuclei in response to acute and chronic osmotic challenges by using this transgenic rat line (Fujihara et al. 2009; Yoshimura et al. 2013) (Fig. 4c–c”““, d–d”““, e–e”““, f). The induction of c-*fos*-mRFP1 was also upregulated by nociceptive stimuli (Ishikura et al. 2012). According to our previous study, the c-*fos*-mRFP1 expression may be maintained longer than the endogenous c-*fos* in the spinal cord and the PVN (Ishikura et al. 2012).

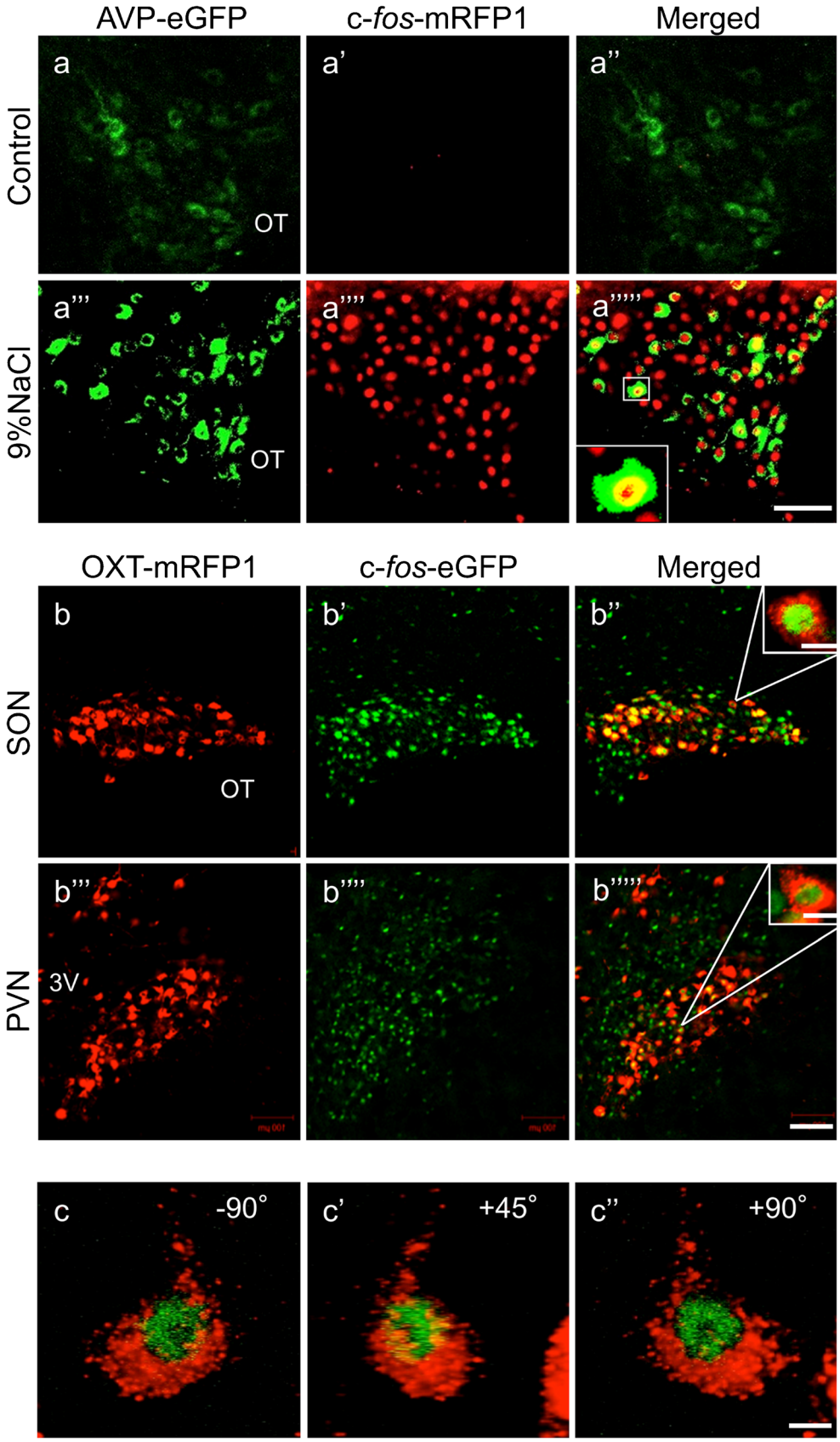
As with AVP-eGFP and OXT-mRFP-1 double transgenic rats, we are able to generate double transgenic AVP-eGFP and c-*fos*-mRFP1 rats by simply cross breeding them (Fujihara et al. 2009). In these transgenic rats, we were able to simultaneously observe increased intensities of both mRFP1 and eGFP after acute osmotic challenge in the SON and PVN (Fujihara et al. 2009) (Fig. 5a–a”“’).

Double transgenic rats, which express both c-*fos*-eGFP and OXT-mRFP1, were also generated (Katoh et al. 2014). Nuclear eGFP appeared in OXT-mRFP1 neurones in the SON and PVN 90 min after i.p. administration of CCK-8. In these neurones, abundant OXT granules in the cytoplasm could clearly be visible by an image obtained from a higher magnification of confocal laser microscopy (Katoh et al. 2014) (Fig. 5b–b”“’).

In these double transgenic rats, neurones activated by a specific neurochemical identity can be readily detected. It would also be possible that 3D reconstruction image may enable us to observe the vesicle dynamics in the cytoplasm in live cells (Fig. 5c–c”). These transgenic rats are particularly valuable tools for visualizing activation of AVP and OXT neurones, especially for live imaging or electrophysiological studies after physiological stimulation.

Novel technology to manipulate AVP and OXT neurone activity

Rapid development of chemogenetic and optogenetic technology has been a great advance in the field of neuroscience (Deisseroth 2011; Magistretti and Allaman 2013). These techniques have been used to understand the linkage between central nervous activity and behaviors. Optogenetics uses light-sensitive channels such as channel rhodopsin 2 (ChR2) so that neurones expressing these channels can be manipulated by light. Chemogenetics uses molecularly engineered receptors and exogenous ligands that are specific for those receptors, also known as designer receptors exclusively activated by designer drugs (DREADDs), to activate only neurones expressing the “designer receptors.” Human muscarinic acetylcholine receptor (hM3Dq) is one of the pharmacologically modulated G protein coupled receptors (GPCRs) which enables us to exploit the Gq signaling pathway. Clozapine-N-oxide (CNO), which is a ligand of hM3Dq, activates Gq-mediated signaling. Recently, chemogenetics seems to be favored over optogenetics because of its spatial resolution,


AVP-eGFP
c-fos-mRFP1
Merged
Control
9%NaCl
a
a'
a''
a'''
a''''
a'''''
OT
OT
OXT-mRFP1
c-fos-eGFP
Merged
SON
PVN
b
b'
b''
b'''
b''''
b'''''
OT
3V
c
c'
c''
-90°
+45°
+90°

◀ **Fig. 5** Neuronal activation visualized by generating double transgenic rat. Endogenous AVP-GFP (**a**, **a**”’) and c-*fos*-mRFP1 expression (**a’**, **a**”“) after intraperitoneal administration of 0.9% saline (**a**–**a**”) or 9% hypertonic saline (**a**”’–**a**”“’) in the SON obtained from an AVP-eGFP and c-*fos*-mRFP1 double transgenic rat. Merged images (**a**”, **a**”“’) are shown. Scale bar 40 μm. *OT* optic tract. Endogenous OXT-mRFP1 (**b**, **b**”’) and c-*fos*-eGFP expression (**b’**, **b**”“) 90 min after intraperitoneal administration of cholecystokinin (CCK)-8 in the SON (**b**–**b**”) and PVN (**b**”’–**b**”“’) obtained from an OXT-mRFP1 and c-*fos*-eGFP double transgenic rat. Merged images are represented (**b**”, **b**”“’). Scale bars 10 μm in lower, and 40 μm in higher magnification image. Three-dimensional (3D) reconstruction images with confocal laser scanning microscopy (**c**–**c**”). Images in these panels are obtained from a same neurone in an OXT-mRFP1 and c-*fos*-eGFP double transgenic rat. Scale bar 10 μm. Reproduced modification from ref. Fujihara et al. (2009) Robust upregulation of nuclear red fluorescent-tagged fos marks neuronal activation in green fluorescent vasopressin neurons after osmotic stimulation in a double transgenic rat. *Endocrinology* 150:5633–5638, Fig. No. 4 with permission of the Endocrine Society and ref. Katoh et al. (2014) Fluorescent visualization of the hypothalamic oxytocin neurones activated by cholecystokinin-8 in rats expressing c-fos-enhanced green fluorescent protein and oxytocin-monomeric red fluorescent protein 1 fusion transgenes. *J Neuroendocrinol* 26, Fig. No. 3 and 4 with permission of John Wiley and Sons

though optogenetics is still useful for investigating functional neuronal circuitry. Light scattering or the distance between the targeted neurones and the light source do not always allow us to activate all of the neurones which express the light-sensitive channels. However, chemogenetics compensate for these disadvantages and therefore would achieve a higher spatial resolution (Roth 2016).

To date, we have generated two transgenic rat lines, AVP-ChR2-eGFP and AVP-hM3Dq-mCherry, for use in optogenetic and chemogenetic experiments, respectively (Ishii et al. 2016; Yoshimura et al. 2017). In contrast to our eGFP and mRFP1 fusion genes with AVP, OXT, or c-*fos*, which produce fusion proteins, we used only promotor sequences of AVP in the AVP-ChR2-eGFP and AVP-hM3Dq-mCherry transgenic rat lines. SV40 poly A sequences were framed to the ChR2-eGFP and hM3Dq-mCherry sequences just before the exon of AVP. This means that AVP is not produced from the transgene, but instead ChR2-eGFP or hM3Dq-mCherry protein is produced under the control of the endogenous AVP promoter. All of our transgenic rat lines

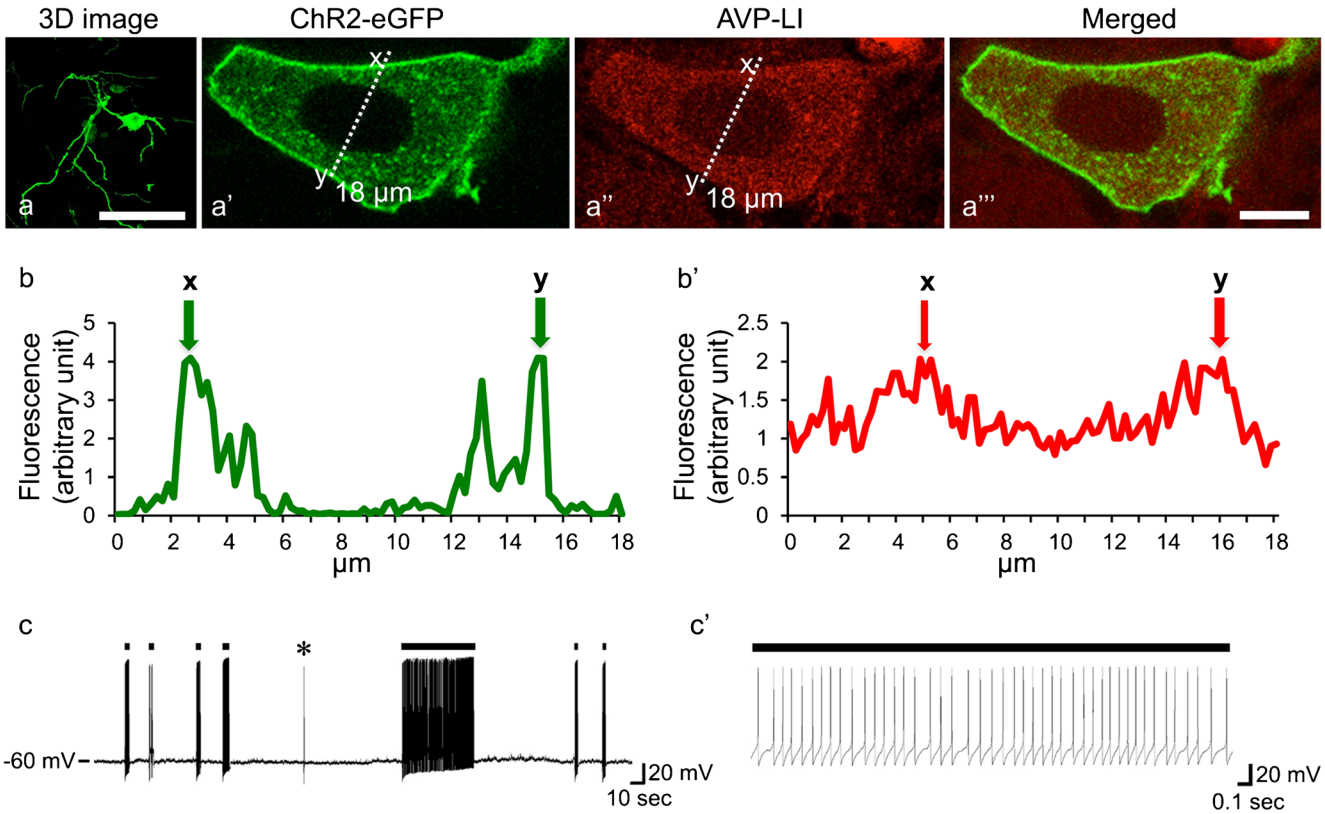


Fig. 6 Optogenetic approach to manipulate AVP neurones’ activity. Example of a Channel rhodopsin (ChR2)-eGFP neurone in the paraventricular nucleus (PVN) obtained from an AVP-ChR2-eGFP transgenic rat line (**a**–**a**”’). ChR2-eGFP is located mainly in the membrane (**a’**, **b**), while AVP-like immunoreactivity (LI) is distributed diffusely in the cytoplasm (**a**”, **b’**). Merged image is also represented (**a**”’). The fluorescence intensity profiles of ChR2-eGFP and AVP-LI were measured at the location of dashed line (x–y), respectively (**b**, **b’**). Whole-cell patch cramp recording from a neurone obtained from the SON (**c**, **c’**). Blue light evoked action potentials with depolarization in current clamp condition. Black bars indicate the time when blue light was on. Asterisk indicates spontaneous action potential. Reproduced modification from ref. Ishii et al. (2016) Transgenic approach to express the channelrhodopsin 2 gene in arginine vasopressin neurons of rats. *Neurosci Lett* 630, Fig. No. 2 and 3 with permission of the Elsevier

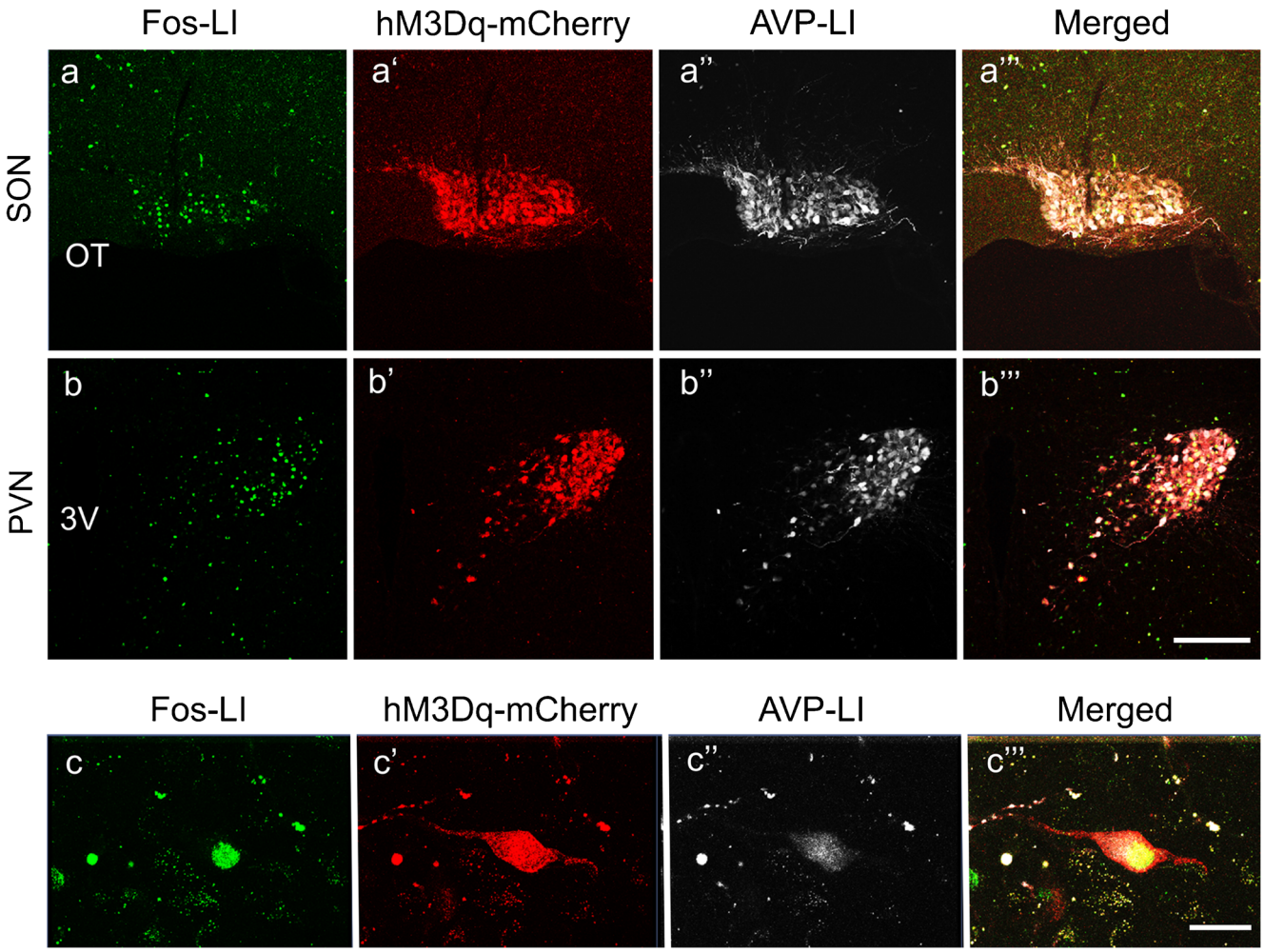


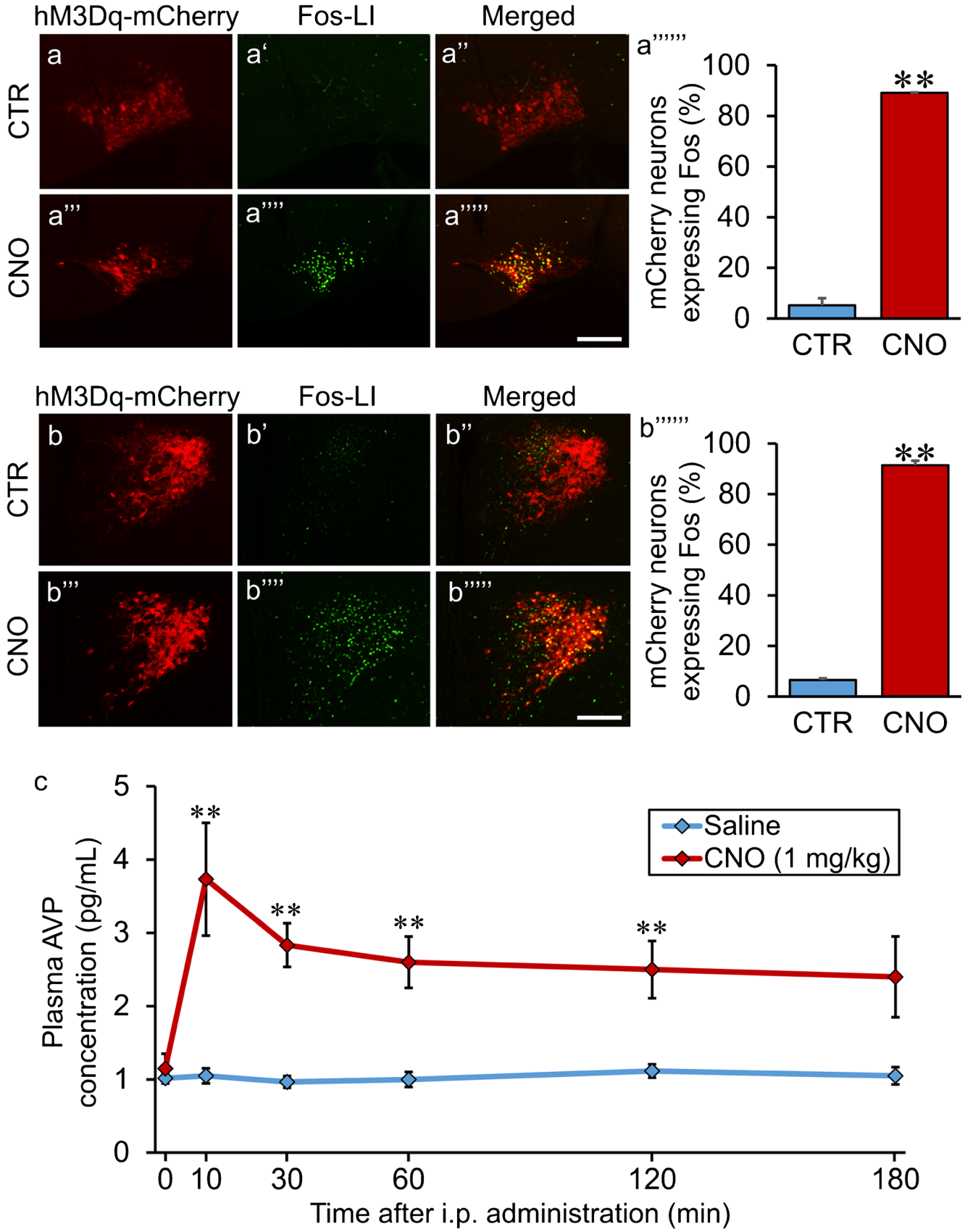
Fig. 7 Chemogenetic approach to manipulate AVP neurones' activity. Digital images of immunohistochemistry for Fos (**a, b**) and AVP (**a**", **b**") in the SON (**a**–**a**"') and PVN (**b**–**b**"') 90 min after intraperitoneal administration of clozapine-N-oxide (CNO) (1 mg/kg) obtained from an AVP-hM3Dq-mCherry transgenic rat line. Endogenous hM3Dq-mCherry can be observed (**a'**, **b'**). Merged image are demonstrated (**a**"', **b**"'). Scale bar, 200 μm. *OT* optic tract, *3rd V* third ventricle. Reconstructed 3D images of an AVP neuron in the PVN 90 min after i.p. administration of CNO (1 mg/kg) obtained from an AVP-hM3Dq-mCherry transgenic rat line (**c**–**c**"'). Fos-LI was observed as green round shaped nucleus (**c**). Endogenous hM3Dq-mCherry is expressed mainly around the membrane of a neurone and dendrite (**c'**), whereas AVP-LI is expressed diffusely in the cytoplasm (**c**"). Merged image is represented (**c**"'). Scale bar, 20 μm

are maintained as heterozygotes since it may lead to being lethal or infertile if they are maintained as homozygous.

In the AVP-ChR2-eGFP transgenic rat line, the expression of ChR2-eGFP was specific in the SON and PVN which are known to contain AVP neurones. ChR2-eGFP was densely localized in the membrane in AVP neurones (Fig. 6a–a"', b–b'). This indicates that ChR2-eGFP could be transported to the plasma membrane to form functional channels. Blue light (435~480 nm of wave length) evoked action potentials repeatedly in ChR2-eGFP neurones that were dissociated from the SON (Ishii et al. 2016) (Fig. 6c–c').

Vivid mCherry fluorescence could be observed in the suprachiasmatic nucleus (SCN), SON, and PVN where AVP neurones are localized in the AVP-hM3Dq-mCherry transgenic rat line. Fos-like-immunoreactivity (-LI) was induced in the hM3Dq-mCherry neurones in the SON and PVN after intraperitoneally administered CNO (1 mg/kg). AVP-LI was completely merged with mCherry neurones in the SON and

Fig. 8 Activation of AVP neurones after CNO administration. ▶ Endogenous hM3Dq-mCherry (**a**, **a"'**, **b**, **b"'**), Fos-LI neurons (**a'**, **a"'**, **b'**, **b"'**), and their merged images (**a"'**, **a"'**, **b"'**, **b"'**) of the SON (**a**–**a"'**) and PVN (**b**–**b"'**) are displayed that are obtained from an AVP-hM3Dq-mCherry transgenic rat line. The group given saline as control (CTR) (**a**–**a"**, **b**–**b"**) or CNO (1 mg/kg) (**a"'**–**a"'**, **b"'**–**b"'**) were compared 90 min after intraperitoneal administration of each compound. The percentages of hM3Dq-mCherry neurons expressing Fos-LI in the SON were counted manually (**a"'**, **b"'**). Scale bars, 100 μm. $^{**}P < 0.01$ vs. CTR. Data are presented as means ± SEM ($n = 6$, each). Plasma AVP concentration was increased at 10, 30, 60, and 120 min after intraperitoneal administration of CNO (1 mg/kg) compared to saline ($n = 4$–6 in each group at each time point) in an AVP-hM3Dq-mCherry transgenic rat line (**c**). $^{**}P < 0.01$ vs. CTR. Data are presented as means ± SEM


hM3Dq-mCherry
Fos-LI
Merged
CTR
CNO
a
a‘
a”
a”’
a””
a”””
a’’’’’’
mCherry neurons expressing Fos (%)
100
80
60
40
20
0
**
CTR
CNO
b
b’
b”
b”’
b””
b”””
b’’’’’’
c
Plasma AVP concentration (pg/mL)
5
4
3
2
1
0
Saline
CNO (1 mg/kg)
0
10
30
60
120
180
Time after i.p. administration (min)

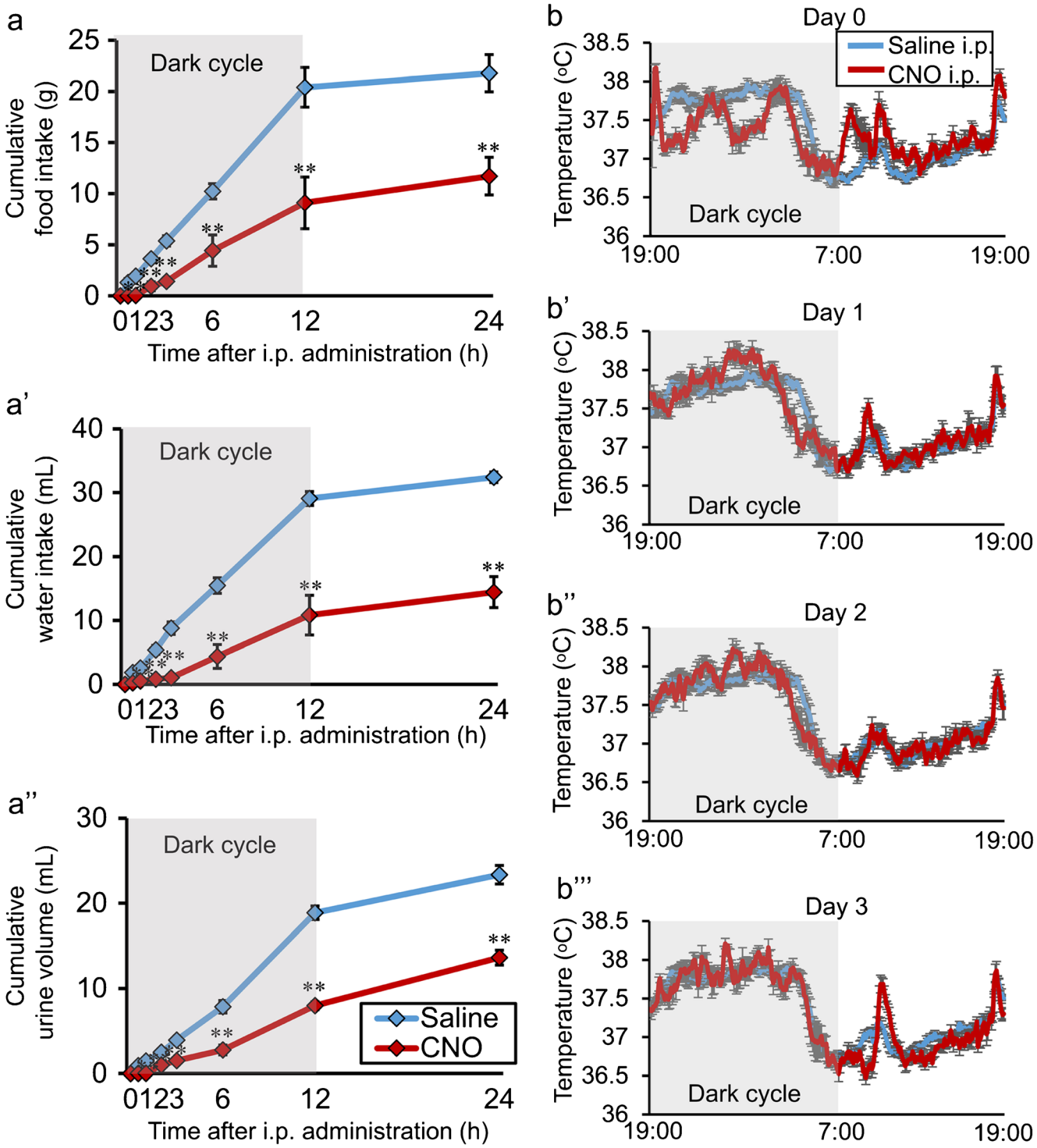


Fig. 9 Behavioral change after activation of AVP neurones by chemogenetics. Cumulative food intake (**a**), water intake (**a'**), and urine volume (**a''**) was significantly decreased after intraperitoneal administration of CNO (1 mg/kg) compared with saline in an AVP-hM3Dq-mCherry transgenic rat line. Data are presented as means ± SEM ($n = 6$, each). $^{**}P < 0.01$ vs. saline. Core body temperature was disregulated after intraperitoneal administration of CNO (1 mg/kg) in an AVP-hM3Dq-mCherry transgenic rat line (**b**–**b'''**). Data are presented as mean ± SEM ($n = 5$, each)

PVN, indicating that these hM3Dq-mCherry neurones are functioning and specific to AVP neurones (Fig. 7a–a''', b–b''', c–c'''). About 90% of mCherry expressed Fos 90 min after CNO (1 mg/kg) administration (Fig. 8a–a''''', b–b'''''). Plasma AVP concentration was significantly increased until 120 min after intraperitoneal administration of CNO (1 mg/kg)

(Fig. 8c). Strikingly, food intake, water intake, and urine volume were dramatically decreased after activation of AVP neurones (Fig. 9a–a"). Locomotor activity and body temperature were also disturbed, thus circadian rhythm could be disrupted after endogenous AVP activation (Yoshimura et al. 2017) (Fig. 9b–b"'). It is possible that the food intake, in rats treated with CNO, could be decreased as a result of the decreased water intake. There is an interesting report that AVP neurones in the PVN regulate food intake via the melanocortin pathway (Pei et al. 2014). Dysregulation of the circadian rhythmicity could also affect food intake (Yamaguchi 2018). We have not examined how and which nucleus/nuclei of AVP altered food intake. However, because all of AVP neurones might be activated after CNO injection in our DREADDs transgenic rat line, both of these mechanisms might affect food intake.

These transgenic rat lines provide us with a novel way to interrogate neuronal circuits in the rat brain and explore neuronal function both in ex vivo and in vivo cultures. Our future goal is to further develop the present transgenic techniques for optogenetic or chemogenetic in vivo and ex vivo experiments on the role of AVP-mediated signaling in behavioral control. We are also currently working on the generation of further novel transgenic rat lines, for example, in which we can control the OXT neurones by using chemogenetic technique.

Perspective and conclusion

The application of transgenes is a rapidly growing area of molecular biology. It is predicted that over 300,000 lines of transgenic animals will be generated (Houdebine 2005). It could be said that the potential to treat genetic diseases by using transgenic animals is one of the most promising applications of transgenes. In the study of neuroscience, much of the function of proteins or genes, individual difference, and interaction of proteins and genes have been unveiled through dramatic progress made in the past decades. To elucidate mechanisms underlying function, the technical demands for labelling or manipulating specific neurones will certainly increase. From this perspective, optogenetic and chemogenetic approaches may be one of the most powerful new tools for the study of neuroscience.

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Author contribution M.Y. prepared the draft and figures. Final approval was made by Y.U.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

References

Agarwal S, Kanaar R, Essers J (2007) The cell biology of homologous recombination. In: Molecular genetics of recombination, pp 335–362

Akagi K, Sandig V, Vooijs M et al (1997) Cre-mediated somatic site-specific recombination in mice. Nucleic Acids Res 25:1766–1773. https://doi.org/10.1093/nar/25.9.1766

Arase K, Hashimoto H, Sonoda S et al (2017) Possible involvement of central oxytocin in cisplatin-induced anorexia in rats. J Physiol Sci. https://doi.org/10.1007/s12576-017-0550-z

Armario A (2006) The contribution of immediate early genes to the understanding of brain processing of stressors. In: Immediate early genes in sensory processing, cognitive performance and neurological disorders, pp 199–221

Armstrong WE, Smith BN, Tian M (1994) Electrophysiological characteristics of immunochemically identified rat oxytocin and vasopressin neurones in vitro. J Physiol 475:115–128. https://doi.org/10.1113/jphysiol.1994.sp020053

Bostick B, Ghosh A, Yue Y et al (2007) Systemic AAV-9 transduction in mice is influenced by animal age but not by the route of administration. Gene Ther 14:1605–1609. https://doi.org/10.1038/sj.gt.3303029

Brinster RL, Chen HY, Trumbauer M et al (1981) Somatic expression of herpes thymidine kinase in mice following injection of a fusion gene into eggs. Cell 27:223–231. https://doi.org/10.1016/0092-8674(81)90376-7

Brownstein MJ, Russell JT, Gainer H (1980) Synthesis, transport, and release of posterior pituitary hormones. Science 207:373–378. https://doi.org/10.1126/science.6153132

Burns KH, Boeke JD (2012) Human transposon tectonics. Cell 149:740–752

Christian M, Cermak T, Doyle EL et al (2010) Targeting DNA double-strand breaks with TAL effector nucleases. Genetics 186:756–761. https://doi.org/10.1534/genetics.110.120717

Cockrell AS, Kafri T (2007) Gene delivery by lentivirus vectors. Mol Biotechnol 36:184–204

Costantini F, Lacy E (1981) Introduction of a rabbit β-globin gene into the mouse germ line. Nature 294:92–94. https://doi.org/10.1038/294092a0

de Bartolomeis A, Buonaguro EF, Latte G et al (2017) Immediate-early genes modulation by antipsychotics: translational implications for a putative gateway to drug-induced long-term brain changes. Front Behav Neurosci 11. https://doi.org/10.3389/fnbeh.2017.00240

de Kock CPJ, Wierda KDB, Bosman LWJ et al (2003) Somatodendritic secretion in oxytocin neurons is upregulated during the female reproductive cycle. J Neurosci 23:2726–2734

Deisseroth K (2011) Optogenetics. Nat Methods 8:26–29. https://doi.org/10.1038/nmeth.f.324

Eliava M, Melchior M, Knobloch-Bollmann HS et al (2016) A new population of parvocellular oxytocin neurons controlling magnocellular neuron activity and inflammatory pain processing. Neuron 89:1291–1304. https://doi.org/10.1016/j.neuron.2016.01.041

Fields RL, Ponzio TA, Kawasaki M, Gainer H (2012) Cell-type specific oxytocin gene expression from AAV delivered promoter deletion constructs into the rat supraoptic nucleus in vivo. PLoS One 7. https://doi.org/10.1371/journal.pone.0032085

Fujihara H, Ueta Y, Suzuki H et al (2009) Robust up-regulation of nuclear red fluorescent-tagged fos marks neuronal activation in green fluorescent vasopressin neurons after osmotic stimulation in a double-transgenic rat. Endocrinology 150:5633–5638. https://doi.org/10.1210/en.2009-0796

Fujio T, Fujihara H, Shibata M et al (2006) Exaggerated response of arginine vasopressin-enhanced green fluorescent protein fusion gene to salt loading without disturbance of body fluid homeostasis in rats. J Neuroendocrinol 18:776–785. https://doi.org/10.1111/j.1365-2826.2006.01476.x

Gasiunas G, Barrangou R, Horvath P, Siksnys V (2012) Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci 109:E2579–E2586. https://doi.org/10.1073/pnas.1208507109

Gordon J, Ruddle F (1981) Integration and stable germ line transmission of genes injected into mouse pronuclei. Science 214:1244–1246. https://doi.org/10.1126/science.6272397

Grund T, Goyon S, Li Y et al (2017) Neuropeptide S activates paraventricular oxytocin neurons to induce anxiolysis. J Neurosci 37:12214–12225. https://doi.org/10.1523/JNEUROSCI.2161-17.2017

Hacein-bey-abina S (2002) Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. Engl J Med 346

Hamlet MRJ, Yergeau DA, Kuliyev E et al (2006) Tol2 transposon-mediated transgenesis in Xenopus tropicalis. Genesis 44:438–445. https://doi.org/10.1002/dvg.20234

Harris JA (1998) Using c-fos as a neural marker of pain. Brain Res Bull 45:1–8

Hemantha KD (2014) Transposon mutagenesis. In: Pseudomonas methods and protocols, pp 271–279

Herrera DG, Robertson HA (1996) Activation of c-fos in the brain. Prog Neurobiol 50:83–107

Holmes CL, Russell JA (2004) Vasopressin. Semin Respir Crit Care Med 25:705–711

Houdebine LM (2005) Use of transgenic animals to improve human health and animal production. Reprod Domest Anim:269–281

Iijima N, Miyamoto S, Matsumoto K et al (2017) Development of an imaging system for in vivo real-time monitoring of neuronal activity in deep brain of free-moving rats. Histochem Cell Biol 148:289–298. https://doi.org/10.1007/s00418-017-1576-2

Ishii M, Hashimoto H, Ohkubo J-I et al (2016) Transgenic approach to express the channelrhodopsin 2 gene in arginine vasopressin neurons of rats. Neurosci Lett 630. https://doi.org/10.1016/j.neulet.2016.08.001

Ishikura T, Suzuki H, Yoshimura M et al (2012) Expression of the c-fos-monomeric red fluorescent protein 1 fusion gene in the spinal cord and the hypothalamic paraventricular nucleus in transgenic rats after nociceptive stimulation. Brain Res 1479. https://doi.org/10.1016/j.brainres.2012.08.033

Ivics Z, Li MA, Mátés L et al (2009) Transposon-mediated genome manipulation in vertebrates. Nat Methods 6:415–422

Iwanaga M, Ohno M, Katoh A et al (2011) Upregulation of arginine vasopressin synthesis in the rat hypothalamus after kainic acid-induced seizures. Brain Res 1424:1–8. https://doi.org/10.1016/j.brainres.2011.09.030

Jaenisch R, Mintz B (1974) Simian virus 40 DNA sequences in DNA of healthy adult mice derived from preimplantation blastocysts injected with viral DNA. Proc Natl Acad Sci 71:1250–1254. https://doi.org/10.1073/pnas.71.4.1250

Jinek M, Chylinski K, Fonfara I et al (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337:816–821. https://doi.org/10.1126/science.1225829

Katoh A, Fujihara H, Ohbuchi T et al (2011) Highly visible expression of an oxytocin-monomeric red fluorescent protein 1 fusion gene in the hypothalamus and posterior pituitary of transgenic rats. Endocrinology 152:2768–2774. https://doi.org/10.1210/en.2011-0006

Katoh A, Shoguchi K, Matsuoka H et al (2014) Fluorescent visualisation of the hypothalamic oxytocin neurones activated by cholecystokinin-8 in rats expressing c-fos-enhanced green fluorescent protein and oxytocin-monomeric red fluorescent protein 1 fusion transgenes. J Neuroendocrinol 26. https://doi.org/10.1111/jne.12150

Kim TK, Eberwine JH (2010) Mammalian cell transfection: the present and the future. Anal Bioanal Chem 397:3173–3178. https://doi.org/10.1007/s00216-010-3821-6

Knobloch HS, Charlet A, Hoffmann L et al (2012) Evoked axonal oxytocin release in the central amygdala attenuates fear response. Neuron 73:553–566. https://doi.org/10.1016/j.neuron.2011.11.030

Kolb AF, Siddell SG (1996) Genomic targeting with an MBP-Cre fusion protein. Gene 183:53–60. https://doi.org/10.1016/S0378-1119(96)00470-2

Kortus S, Srinivasan C, Forostyak O et al (2016) Physiology of spontaneous [Ca2+]i oscillations in the isolated vasopressin and oxytocin neurones of the rat supraoptic nucleus. Cell Calcium 59:280–288. https://doi.org/10.1016/j.ceca.2016.04.001

Leon WC, Canneva F, Partridge V et al (2010) A novel transgenic rat model with a full Alzheimer's-like amyloid pathology displays pre-plaque intracellular amyloid-beta-associated cognitive impairment. J Alzheimers Dis 20:113–126. https://doi.org/10.3233/JAD-2010-1349

Ludwig M, Leng G (2006) Dendritic peptide release and peptide-dependent behaviours. Nat Rev Neurosci 7:126–136. https://doi.org/10.1038/nrn1845

Magistretti PJ, Allaman I (2013) Chemogenetics: DREADDs. In: Neuroscience in the 21st century, pp 1591–1620

Marshall K (2011) Oxytocin. In: xPharm: the comprehensive pharmacology reference, pp 1–6

Matsuura T, Kawasaki M, Hashimoto H et al (2015) Fluorescent visualisation of oxytocin in the hypothalamo-neurohypophysial/-spinal pathways after chronic inflammation in oxytocin-monomeric red fluorescent protein 1 transgenic rats. J Neuroendocrinol 27. https://doi.org/10.1111/jne.12290

Matsuura T, Kawasaki M, Hashimoto H et al (2016) Possible involvement of the rat hypothalamo-neurohypophysial/-spinal oxytocinergic pathways in acute nociceptive responses. J Neuroendocrinol 28. https://doi.org/10.1111/jne.12396

Meek S, Mashimo T, Burdon T (2017) From engineering to editing the rat genome. Mamm Genome 28:302–314. https://doi.org/10.1007/s00335-017-9705-8

Menon R, Grund T, Zoicas I et al (2018) Oxytocin signaling in the lateral septum prevents social fear during lactation. Curr Biol 28:1066–1078.e6. https://doi.org/10.1016/j.cub.2018.02.044

Morbitzer R, Romer P, Boch J, Lahaye T (2010) Regulation of selected genome loci using de novo-engineered transcription activator-like effector (TALE)-type transcription factors. Proc Natl Acad Sci 107:21617–21622. https://doi.org/10.1073/pnas.1013133107

Morgan JI, Cohen DR, Hempstead JL, Curran T (1987) Mapping patterns of c-fos expression in the central nervous system after seizure. Science 237:192–197. https://doi.org/10.1126/science.3037702

Motojima Y, Kawasaki M, Matsuura T et al (2016) Effects of peripherally administered cholecystokinin-8 and secretin on feeding/drinking and oxytocin-mRFP1 fluorescence in transgenic rats. Neurosci Res 109:63–69. https://doi.org/10.1016/j.neures.2016.02.005

Motojima Y, Matsuura T, Yoshimura M et al (2017) Comparison of the induction of c-fos-eGFP and Fos protein in the rat spinal cord and hypothalamus resulting from subcutaneous capsaicin or formalin injection. Neuroscience 356. https://doi.org/10.1016/j.neuroscience.2017.05.015

Nagy A (2000) Cre recombinase: the universal reagent for genome tailoring. Genesis 26:99–109

Ohbuchi T, Sato K, Suzuki H et al (2010a) Acid-sensing ion channels in rat hypothalamic vasopressin neurons of the supraoptic nucleus. J Physiol 588:2147–2162. https://doi.org/10.1113/jphysiol.2010.187625

Ohbuchi T, Ueta Y (2014) Visible markers of vasopressin and oxytocin activity and their use in identifying the neuronal activity of specific neuroendocrine cell types. In: Neurophysiology of Neuroendocrine Neurons, pp 135–162

Ohbuchi T, Yokoyama T, Fujihara H et al (2010b) Electrophysiological identification of the functional presynaptic nerve terminals on an isolated single vasopressin neurone of the rat supraoptic nucleus. J Neuroendocrinol 22:413–419. https://doi.org/10.1111/j.1365-2826.2010.01979.x

Ohbuchi T, Yokoyama T, Saito T et al (2009) Brain-derived neurotrophic factor inhibits spontaneous inhibitory postsynaptic currents in the rat supraoptic nucleus. Brain Res 1258:34–42. https://doi.org/10.1016/j.brainres.2008.12.057

Ohkubo J-I, Ohbuchi T, Yoshimura M et al (2014a) Differences in acid-induced currents between oxytocin-mRFP1 and vasopressin-eGFP neurons isolated from the supraoptic and paraventricular nuclei of transgenic rats. Neurosci Lett 583. https://doi.org/10.1016/j.neulet.2014.09.004

Ohkubo J, Ohbuchi T, Yoshimura M et al (2014b) Electrophysiological effects of kainic acid on vasopressin-enhanced green fluorescent protein and oxytocin-monomeric red fluorescent protein 1 neurones isolated from the supraoptic nucleus in transgenic rats. J Neuroendocrinol 26. https://doi.org/10.1111/jne.12128

Ohno M, Fujihara H, Iwanaga M et al (2012) Induction of arginine vasopressin-enhanced green fluorescent protein expression in the locus coeruleus following kainic acid-induced seizures in rats. Stress 15:435–442. https://doi.org/10.3109/10253890.2011.637185

Ohno S, Hashimoto H, Fujihara H et al (2017) Increased oxytocin-monomeric red fluorescent protein 1 fluorescent intensity with urocortin-like immunoreactivity in the hypothalamo-neurohypophysial system of aged transgenic rats. Neurosci Res

Pei H, Sutton AK, Burnett KH et al (2014) AVP neurons in the paraventricular nucleus of the hypothalamus regulate feeding. Mol Metab 3:209–215. https://doi.org/10.1016/j.molmet.2013.12.006

Persic L, Righi M, Roberts A et al (1997) Targeting vectors for intracellular immunisation. Gene 187:1–8. https://doi.org/10.1016/S0378-1119(96)00627-0

Pfeifer A, Lim T, Zimmermann K (2010) Lentivirus transgenesis. Methods Enzymol 477:3–15. https://doi.org/10.1016/S0076-6879(10)77001-4

Pfeifer A, Verma IM (2001) Gene therapy: promises and problems. Annu Rev Genomics Hum Genet 2:177–211. https://doi.org/10.1146/annurev.genom.2.1.177

Ponzio TA, Fields RL, Rashid OM et al (2012) Cell-type specific expression of the vasopressin gene analyzed by AAV mediated gene delivery of promoter deletion constructs into the rat SON in vivo. PLoS One 7. https://doi.org/10.1371/journal.pone.0048860

Popova E, Bader M, Krivokharchenko A (2005) Production of transgenic models in hypertension. Methods Mol Med 108:33–50

Roth BL (2016) DREADDs for neuroscientists. Neuron 89:683–694

Sasakura Y, Oogai Y, Matsuoka T et al (2007) Transposon mediated transgenesis in a marine invertebrate chordate: Ciona intestinalis. Genome Biol 8

Shashikant CS, JL. Carr, Bhargava J et al (1998) Recombinogenic targeting: a new approach to genomic analysis—a review. In: Gene, pp 9–20

Shi P, Martinez MA, Calderon AS et al (2008) Intra-carotid hyperosmotic stimulation increases Fos staining in forebrain organum vasculosum laminae terminalis neurones that project to the hypothalamic paraventricular nucleus. J Physiol 586:5231–5245. https://doi.org/10.1113/jphysiol.2008.159665

Shibata M, Fujihara H, Suzuki H et al (2007) Physiological studies of stress responses in the hypothalamus of vasopressin-enhanced green fluorescent protein transgenic rat. J Neuroendocrinol 19:285–292. https://doi.org/10.1111/j.1365-2826.2007.01532.x

Suzuki H, Kawasaki M, Ohnishi H et al (2009) Exaggerated response of a vasopressin-enhanced green fluorescent protein transgene to nociceptive stimulation in the rat. J Neurosci 29:13182–13189. https://doi.org/10.1523/JNEUROSCI.2624-09.2009

Technologies T (2012) Technical reference guide an introduction to transfection methods. Infection 49:395–401. https://doi.org/10.3791/5068

Terleph TA, Tremere LA (2006) The use of immediate early genes as mapping tools for neuronal activation: concepts and methods. In: Immediate early genes in sensory processing, cognitive performance and neurological disorders, pp 1–10

Tobin VA, Hashimoto H, Wacker DW et al (2010) An intrinsic vasopressin system in the olfactory bulb is involved in social recognition. Nature 464:413–417. https://doi.org/10.1038/nature08826

Trudeau DL, Smith MA, Arnold FH (2013) Innovation by homologous recombination. Curr Opin Chem Biol 17:902–909

Tsien R (1998) The green fluorescent protein. Annu Rev Biochem 67:509. https://doi.org/10.1146/annurev.biochem.67.1.509

Tsuji T, Allchorne AJ, Zhang M et al (2017) Vasopressin casts light on the suprachiasmatic nucleus. J Physiol 595:3497–3514. https://doi.org/10.1113/JP274025

Ueta Y, Fujihara H, Serino R et al (2005) Transgenic expression of enhanced green fluorescent protein enables direct visualization for physiological studies of vasopressin neurons and isolated nerve terminals of the rat. Endocrinology 146:406–413. https://doi.org/10.1210/en.2004-0830

Urnov FD, Miller JC, Lee YL et al (2005) Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nature 435:646–651. https://doi.org/10.1038/nature03556

von Horsten S, Schmitt I, Nguyen HP et al (2003) Transgenic rat model of Huntington's disease. Hum Mol Genet 12:617–624. https://doi.org/10.1093/hmg/ddg075

Vorburger SA, Hunt KK (2002) Adenoviral gene therapy. Oncologist 7. https://doi.org/10.1634/theoncologist.7-1-46

Woods NB, Muessig A, Schmidt M et al (2003) Lentiviral vector transduction of NOD/SCID repopulating cells results in multiple vector integrations per transduced cell: risk of insertional mutagenesis. Blood 101:1284–1289. https://doi.org/10.1182/blood-2002-07-2238

Yamaguchi Y (2018) Arginine vasopressin signaling in the suprachiasmatic nucleus on the resilience of circadian clock to jet lag. Neurosci Res 129:57–61. https://doi.org/10.1016/j.neures.2017.10.007

Yamashita H, Ueta Y, Dyball REJ (2002) 60 - Electrophysiological and molecular properties of the oxytocin- and vasopressin-secreting systems in mammals. In: Hormones, brain and behavior, pp 1–49

Yoshimura M, Matsuura T, Ohkubo J et al (2014) A role of nesfatin-1/NucB2 in dehydration-induced anorexia. Am J Phys Regul Integr Comp Phys 307:R225–R236. https://doi.org/10.1152/ajpregu.00488.2013

Yoshimura M, Nishimura K, Nishimura H et al (2017) Activation of endogenous arginine vasopressin neurons inhibit food intake: by using a novel transgenic rat line with DREADDs system. Sci Rep 7. https://doi.org/10.1038/s41598-017-16049-2

Yoshimura M, Ohkubo J, Katoh A et al (2013) A c-fos-monomeric red fluorescent protein 1 fusion transgene is differentially expressed in rat forebrain and brainstem after chronic dehydration and rehydration. J Neuroendocrinol 25. https://doi.org/10.1111/jne.12022

Zeng Q, Foo NC, Funkhouser JM et al (1994) Expression of a rat vasopressin transgene in rat testes. J Reprod Fertil 102:471–481