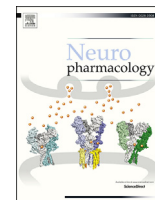




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Invited review

Physiological roles of CNS muscarinic receptors gained from knockout mice

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ABSTRACT

Because the five muscarinic acetylcholine receptor subtypes have overlapping distributions in many CNS tissues, and because ligands with a high degree of selectivity for a given subtype long remained elusive, it has been difficult to determine the physiological functions of each receptor. Genetically engineered knockout mice, in which one or more muscarinic acetylcholine receptor subtype has been inactivated, have been instrumental in identifying muscarinic receptor functions in the CNS, at the neuronal, circuit, and behavioral level. These studies revealed important functions of muscarinic receptors modulating neuronal activity and neurotransmitter release in many brain regions, shaping neuronal plasticity, and affecting functions ranging from motor and sensory function to cognitive processes. As gene targeting technology evolves including the use of conditional, cell type specific strains, knockout mice are likely to continue to provide valuable insights into brain physiology and pathophysiology, and advance the development of new medications for a range of conditions such as Alzheimer's disease, Parkinson's disease, schizophrenia, and addictions, as well as non-opioid analgesics.

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Abbreviations: CIN, cholinergic interneuron; CAMKII α , Ca²⁺/calmodulin-dependent protein kinase II α ; EPSP, excitatory postsynaptic potentials; HPA, hypothalamic-pituitary-adrenocortical; MAPK, mitogen-activated protein kinase; LTD, long-term depression; LTP, long-term potentiation; MSN, medium spiny neurons; mAChR, muscarinic acetylcholine receptor; PAM, positive allosteric modulator; PI, phosphatidylinositol; PPI, prepulse inhibition; SNc, substantia nigra pars compacta; VTA, ventral tegmental area.

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1. Neuronal muscarinic receptor functions

1.1. Neuronal excitability, synaptic transmission, and neurotransmitter release

Unlike its classic neurotransmitter function in the periphery (e.g., at neuromuscular junctions), in the CNS, acetylcholine often functions as a modulator of neuronal activity. Acetylcholine, released from projection neurons and/or local interneurons, increases or decreases neuronal excitability and synaptic release of neurotransmitters, and modulates temporal patterns and coordination of activity between neurons (see [Goldberg et al., 2012](#); [Picciotto et al., 2012](#); [Smythies, 2005](#)). Given the complex and overlapping distribution of nicotinic and muscarinic acetylcholine receptor (mAChR) subtypes across the brain, combined with the difficulty in generating ligands with a high degree of selectivity at each muscarinic receptor subtype, transgenic mice lacking one or more functional mAChR subtypes ($M_1^{-/-}$ to $M_5^{-/-}$ mice) have been invaluable in dissecting these neuronal functions of acetylcholine (previously reviewed in [Gautam et al., 2006](#); [Matsui et al., 2004](#); [Wess, 2012, 2004](#); [Wess et al., 2007](#)). Please see chapter 1 of the special issue, “Central muscarinic cholinergic system” for information about mAChR distribution and general characteristics.

1.1.1. Striatal functions

Acetylcholine in striatal tissues exemplifies cholinergic interneuron (CIN) function: representing only 1–2% of striatal neurons, the tonically active CINs are the major cholinergic input to the region and provide extensive innervation that potently modulate striatal functions ([Gonzales and Smith, 2015](#)). Striatal CINs express G_i -coupled M_2 and M_4 mAChRs ([Bernard et al., 1992](#); [Yan and Surmeier, 1996](#)) and these are proposed to mediate suppression of dopamine transmission in the striatum ([Bonsi et al., 2008](#); [Shin et al., 2015](#); [Zhang et al., 2002a](#)). Initial investigation of dopamine release in slices from mAChR knockout mice suggested that multiple mAChR subtypes are involved in regulation of striatal dopamine, with some receptors (M_3 and M_4) mediating their effects indirectly via modulations of striatal GABA tone ([Zhang et al., 2002b](#)). While the effects of inactivation of different mAChR subtypes on dopamine release have been conflicting ([Bendor et al., 2010](#); [Forster et al., 2002](#); [Foster et al., 2014b](#); [Tzavara, 2004](#); [Yamada et al., 2001](#); [Zhang et al., 2002b](#)), studies applying fast-scan cyclic voltammetry and amperometry have been valuable in the understanding of these, and conclude that a key action of mAChRs is to modulate acetylcholine tone from CINs ([Shin et al., 2015](#); [Threlfell et al., 2010](#)). Depression of striatal dopamine transmission is proposed to arise from a disinaptic mechanism by which activation of M_2/M_4 autoreceptors on CINs creates an inhibitory outward current and decreased conductance, resulting in decreased cholinergic tone and subsequent nicotinic acetylcholine receptor-dependent dopamine transmission ([Bonsi et al., 2008](#); [Shin et al., 2015](#); [Threlfell et al., 2010](#)). This modulation is not simply inhibitory, but also makes dopamine release more sensitive to the frequency of neuronal firing, and seems to be controlled by M_2 and M_4 mAChRs in the dorsal striatum, but only by M_4 mAChRs in the ventral striatum/nucleus accumbens ([Threlfell et al., 2010](#); [Threlfell and Cragg, 2011](#)). Consistent with this, whole-tissue

striatal dopamine and metabolites were normal in $M_4^{-/-}$ mice ([Dencker et al., 2012b](#)), but $M_4^{-/-}$ mice displayed increased psychostimulant-induced extracellular dopamine efflux in the nucleus accumbens ([Schmidt et al., 2011](#); [Tzavara, 2004](#)). No alteration of accumbal psychostimulant-induced dopamine efflux was detected in $M_2^{-/-}$ mice ([Tzavara, 2004](#)), in agreement with the notion that M_4 , but not M_2 receptor activation exert inhibitory control on evoked dopamine release in ventral striatum.

The dense and extensive axonal branching of CINs results in a widespread release of acetylcholine, which acts locally on cholinergic receptors on striatal output medium spiny neurons (MSN). Postsynaptically, excitability of MSN in response to excitatory and inhibitory inputs is modulated by M_1 receptor activation, via KCNQ potassium channel regulation and endocannabinoid-mediated signaling ([Narushima et al., 2007](#); [Shen, 2005](#)). Several lines of evidence including knockout studies indicate that M_1 receptor stimulation enhances the dendritic excitability and spiking of MSN, making them more “responsive” to corticostriatal input ([Ding et al., 2010](#)). This modulation happens preferentially in the D_2 -expressing indirect pathway MSN, thought to provide the inhibitory, “no-go”, side of striatal output ([Ding et al., 2010](#)). Measured at the level of striatal tissue rather than at the cell level, $M_1^{-/-}$ mice had significantly elevated extracellular dopamine levels despite normal whole-tissue levels (i.e., indicating increased release), and dopamine efflux in response to amphetamine was exacerbated ([Gerber et al., 2001](#)). M_4 mAChRs are densely co-expressed with dopamine D_1 receptors on MSNs ([Ince et al., 1997](#); [Yan et al., 2001](#)), and were suggested to act as a functional antagonist of D_1 receptor-mediated cyclic AMP-dependent signaling pathways ([Jeon et al., 2010](#); [Onali and Olanas, 2002](#); see also section 3). M_4 receptors on MSN were also shown to mediate prolonged suppression of dopamine release, through a cannabinoid CB_2 receptor dependent mechanism ([Foster et al., 2016](#)). Corticostriatal glutamatergic transmission is depressed by stimulation of presynaptic M_4 mAChRs ([Higley et al., 2009](#); [Pakhotin and Bracci, 2007](#); [Pancani et al., 2014](#)). Finally, a specific Ca^{2+} /calmodulin-dependent protein kinase II α (CAMKII α) has been found to bind directly and selectively to the M_4 receptor upon calcium influx and to mediate potentiation of M_4 receptor signaling ([Guo et al., 2010a, 2010b](#)). Thus, overall, activation of striatal M_1 and M_4 receptors modulates dopaminergic signaling towards inhibition.

Striatal tissues receive dopaminergic projections from the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc), in which only the M_5 mAChR subtype has been detected. Stimulation of those M_5 receptors increases neuronal activity and dopamine release, and is necessary for sustained striatal dopamine release ([Forster et al., 2002](#); [Foster et al., 2014b](#)). Dopaminergic VTA and SNc neurons in turn receive input from cholinergic neurons in the laterodorsal pontine tegmental nuclei, which are modulated by inhibitory M_2 and M_4 receptors ([Kohlmeier et al., 2012](#)). In $M_5^{-/-}$ mice, extracellular nucleus accumbens dopamine levels were comparable to wild-type controls at baseline, after depolarization (K^+)-induced release, and after electrical stimulation of the medial forebrain bundle ([Basile et al., 2002](#); [Schmidt et al., 2010](#)). However, $M_5^{-/-}$ mice showed dramatically reduced dopamine efflux after stimulation of the laterodorsal or pedunculopontine tegmental nuclei, and electrically

evoked dopamine release in striatal slices from $M_5^{-/-}$ mice was moderately decreased (Bendor et al., 2010; Forster et al., 2002; Steidl et al., 2011). Morphine-induced increases in striatal dopamine levels were also blunted in $M_5^{-/-}$ mice (Basile et al., 2002; Steidl et al., 2011). M_5 mAChRs are expressed by the dopaminergic neurons within the VTA and SNc, but have not been detected on axons, in the striatum (Vilario et al., 1990; Weiner et al., 1990). Nevertheless, recent studies suggest that M_5 receptors within the striatum may also modulate dopamine release, but findings varied in different experimental approaches, and generally suggested a relatively minor contribution of M_5 receptors relative to other mechanism (Bendor et al., 2010; Foster et al., 2014b; Shin et al., 2015). Recycling of the M_5 mAChRs at dopaminergic terminals has been proposed to be highly important for maintaining function. Bendor et al. (2010) report of a new receptor cycling pathway where interaction of the GTPase activating protein AGAP1 in combination adaptor protein AP-3 is required for endocytic recycling of the M_5 receptor. These effects of M_5 receptor inactivation on dopamine release has led to an interest in the receptor with regard to addiction (see 2.5).

1.1.2. Hippocampal functions

In the hippocampus, synaptically released acetylcholine facilitates learning and memory through cholinergic induction of neural oscillations. Excitatory hippocampal pathways comprised of CA3 and CA1 pyramidal neurons are modulated by mAChRs. Studies using mAChR knockout lines suggest that M_1 and M_4 receptors (and not $M_2/M_3/M_5$) are the major mAChR subtypes responsible for cholinergic modulation of the excitatory hippocampal circuits (Dasari and Gulledge, 2011; Dennis et al., 2016; Kremin et al., 2006). Whole cell recordings from $M_1^{-/-}$ mice have shown reduced or lack of both phasic and tonic cholinergic modulation of CA1 and CA3 pyramidal neurons, while $M_3^{-/-}$ mice only displayed minor effects on tonic modulation (Dasari and Gulledge, 2011; Kremin et al., 2006). In addition, neurons from $M_4^{-/-}$ mice display a reduced suppression of Schaffer-collateral excitatory postsynaptic potentials (EPSPs) by the non-selective cholinergic receptor agonist carbachol (Dasari and Gulledge, 2011). Thus, acetylcholine can modulate pyramidal neuron excitability directly as well as through alterations of synaptic transmission between CA3 and CA1 pyramidal neurons. In line with this, muscarinic-induced hippocampal gamma oscillations in CA3 neuron were absent in $M_1^{-/-}$ mice but normal in M_2 – M_5 knockout lines, and carbachol-induced depression of transmission at excitatory synapses was blunted in $M_4^{-/-}$ mice (Fisahn et al., 2002; Shirey et al., 2008). At 6 months of age, $M_5^{-/-}$ mice showed reduced spontaneous neuronal activity in CA3 pyramidal neurons, but this effect could be secondary to the severe cerebrovascular deficiency that becomes apparent in aging $M_5^{-/-}$ mice (Araya et al., 2006). Measured by in vivo microdialysis, data from mice lacking M_2 , M_3 , M_4 or a combination of those subtypes indicated that several mAChRs play a role in regulating hippocampal acetylcholine release, with the M_2 and M_3 receptors appearing to modulate cholinergic responses to environmental and pharmacological manipulations, although $M_5^{-/-}$ mice and, importantly, $M_1^{-/-}$ mice, were not examined (Tzavara et al., 2006, 2003).

GABAergic hippocampal interneurons also modulate firing frequency, neuronal excitability, and membrane potential oscillations in a cell type dependent manner, through M_1 and M_3 receptors (Cea-del Rio la et al., 2010; 2011). In addition, muscarinic attenuation or amplification of excitatory signals in interneurons occurs in a layer-specific fashion, which appears to depend critically on the differential expression of M_2 receptors (Zheng et al., 2011), further indicating the level of complexity at which this region is regulated by mAChRs. Other hippocampal signaling systems are also modulated by mAChRs. In CA1 pyramidal neurons, cholinergic

activation of extracellular signal-regulated kinase (ERK) 1/2 was shown to occur through stimulation of M_1 receptors, but not M_2 , M_3 , or M_4 receptors (Berkeley et al., 2001). Similarly, mAChR agonist-induced activation of the mitogen-activated protein kinase (MAPK) pathway was virtually abolished, and phosphatidylinositol (PI) hydrolysis was reduced, in cortical cultures from $M_1^{-/-}$ mice (Hamilton and Nathanson, 2001). In hippocampal neuron cultures, postsynaptic M_1 and/or M_3 receptors were also found to mediate release of endocannabinoids from postsynaptic neurons and retrogradely suppress inhibitory synaptic transmission by activating presynaptic cannabinoid CB1 receptors (Fukudome et al., 2004; Ohno-Shosaku et al., 2003). Most investigations have focused on selected subtypes, and it would be useful to compare phenotypes of all M_1 – M_5 knockout mice under identical experimental conditions in order to gain a fuller understanding of mAChR modulation of hippocampal functions.

1.1.3. Cortical and other brain functions

Similar to hippocampal pyramidal neuron, pyramidal neurons in the neocortex (layer 5) also respond to acetylcholine mainly via activation of M_1 receptors, and with only minor involvement of M_3 receptors (Gulledge et al., 2009). M_2 or M_4 knockout lines were not tested. In both cortex and hippocampus, M_1 -mediated cholinergic transmission is suggested to be by volume transmission (Yamasaki et al., 2010). In occipital slices of mouse visual cortex from M_1 – M_5 knockout lines, M_2 and M_4 receptor were demonstrated to facilitate synaptic transmission, while multiple mAChRs seem to mediate depression of synaptic transmission (Kuczewski et al., 2005; see section 2.2). Unlike in the hippocampus (Fisahn et al., 2002; Rouse et al., 2000), muscarinic-agonist mediated inhibition of tonically active voltage dependent potassium channel (M-current) was absent in sympathetic ganglion neurons of $M_1^{-/-}$ mice (Hamilton et al., 1997). This effect may be the basis for the lack of muscarinic agonist-induced seizures in $M_1^{-/-}$ mice (see section 2.1). In addition, muscarinic agonist administration in the Bed nucleus of the stria terminalis produces long lasting reduction of stimulus evoked EPSP amplitude, an effect that has been attributed to M_2 receptors, although only $M_1^{-/-}$ and $M_2^{-/-}M_4^{-/-}$ lines were studied (Guo et al., 2012).

1.2. Neuronal plasticity, LTD, and LTP

Acetylcholine also modulates neuronal plasticity through mAChRs in several brain regions. While studies using knockout mice indicate that several muscarinic receptors are important mediators of neuronal plasticity, their overall contributions remain unclear and appear dependent upon the brain region investigated. One reason for this may be that only selected lines were typically investigated for a given tissue/neuronal population. Systematic investigation of multiple receptors across tissues might reveal more cohesive functions of some subtypes, or might confirm a high level of cell type-dependent complexity.

Striatal MSNs show long-term potentiation (LTP) and long-term depression (LTD) in response to input patterns from glutamatergic afferents. Studies using striatal slices from mAChR knockout mice lacking M_2 and/or M_4 receptors indicate that M_2 and M_4 receptors predominantly play a role in the induction of LTD, and not LTP, probably via their autoreceptor function modulating cholinergic tone (Bonsi et al., 2008). Specifically, loss of the M_4 subtype, in $M_4^{-/-}$ mice or $M_2^{-/-}M_4^{-/-}$ double knockout mice, abolished LTD in MSN, but left LTP intact (Bonsi et al., 2008). LTD could be rescued using the M_1 receptor antagonist pirenzepine, or by depleting endogenous acetylcholine using hemicholinium-3, suggesting that the loss of LTD in the M_4 receptor-deficient mice was caused by increased cholinergic tone,

and at least partly by the resulting increased stimulation of M_1 receptors (Bonsi et al., 2008). Furthermore, pharmacological stimulation of M_4 receptors using the positive allosteric modulator (PAM) VU10010 induced LTD in D_1 -expressing direct-pathway MSN, which was abolished in D_1 -neuron-specific $M_4^{-/-}$ mice (Shen et al., 2015). While LTP itself appeared normal in mice lacking M_2/M_4 receptors, LTP can be reverted to resting levels by low frequency stimulation (synaptic depotentiation), and this effect was abolished in corticostriatal slices from $M_2^{-/-}M_4^{-/-}$ mice (Martella et al., 2009). Thus, in striatal tissue, M_2/M_4 receptors appear necessary for the induction of LTD, and for inhibition/modulation of LTP. However, in nucleus accumbens MSN, the mAChR agonist-induced increase in EPSC amplitude appears dependent upon M_5 receptors (Shin et al., 2015).

The above findings on striatal neuronal plasticity do not necessarily generalize to all brain regions. In mouse hippocampal slices, LTP measured in CA1 was decreased in $M_2^{-/-}$ mice, LTP-enhancement by the non-selective cholinergic receptor agonist carbachol was abolished, and short-term potentiation was abolished (Seeger et al., 2004). In the CA3, $M_2^{-/-}$ mice showed an input-specific effect on LTP, suggesting that M_2 receptors promote LTP and short-term facilitation at associational/commissural fiber inputs and inhibit LTP at mossy fiber inputs with little effect on short-term plasticity (Zheng et al., 2012). Thus, in hippocampal tissue, M_2 receptors appear important for the induction of LTP, consistent with $M_2^{-/-}$ mice showing impairments in memory tasks. For the predominantly post-synaptic subtypes M_1 and M_3 , knockout mouse studies suggest that, at least in the hippocampus, these receptors are involved not in the induction of LTD or LTP per se, but rather, modulate the degree of LTP or LTD in response to cholinergic stimulation (pharmacological or endogenous). LTP induction after electrical stimulation was intact in hippocampal slices from $M_1^{-/-}$ mice, forebrain-specific $M_1^{-/-}$ mice, and $M_3^{-/-}$ mice (Kamsler et al., 2010; Shinoue, 2005). However, while carbachol or electrically evoked acetylcholine release enhanced LTP in wild-type mice and in $M_3^{-/-}$ mice, this effect was abolished in the $M_1^{-/-}$ mice (Anagnostaras et al., 2003; Shinoue, 2005). A combination of pharmacological and knockout approaches demonstrated that M_1 receptor stimulation can increase glutamatergic synaptic transmission in CA1 pyramidal neurons, in a mechanism similar to electrically induced LTP (Dennis et al., 2016). Similarly, studies in mice lacking M_1 receptors in specific brain tissues indicated that CA3 M_1 receptors are needed for presynaptic induction of glutamatergic LTD in hippocampus (Kamsler et al., 2010). In addition to classic cell surface mAChR signaling, recent studies in cultured cortical and hippocampal neurons suggest that intracellular mAChRs, which experiments in knockout mice confirmed to be M_1 , can mediate enhancement of LTP through ERK 1/2 phosphorylation (Anisuzzaman et al., 2013). Finally, both LTP measured at CA3 mossy fiber synapses and short-term plasticity (paired-pulse facilitation of field EPSPs) was decreased in $M_5^{-/-}$ mice, although these effects may be a consequence of pronounced cerebrovascular deficiency that develops in aging $M_5^{-/-}$ mice (Araya et al., 2006; see section 2.3).

In visual cortex slices, low frequency stimulation caused a normal LTD in $M_2^{-/-}M_4^{-/-}$ mice, but theta-burst stimulations failed to induce LTP in the $M_2^{-/-}M_4^{-/-}$ mice (Origlia et al., 2006). However, normal LTP in visual cortex slices from single receptor knockout $M_2^{-/-}$ mice and $M_4^{-/-}$ mice suggests a redundant function of both inhibitory autoreceptors in cortical LTP (Origlia et al., 2006). In the same preparations, deletion of M_1 and/or M_3 receptors had no effect on LTP, but caused abnormal plasticity patterns after low-frequency stimulation that elicited LTD in wild-type controls, including reversal from LTD to LTP (Origlia et al., 2006). Thus, in cortex, LTP appears dependent upon M_2/M_4 receptors, while LTD

appears dependent upon M_1/M_3 receptors (Origlia et al., 2006). In the cerebellum, mAChR stimulation has suppressive effects on parallel fiber LTP, an effect that also appears to be mediated through M_1 and/or M_3 receptors (Rinaldo and Hansel, 2013).

1.3. Caveats and compensatory changes in knockout mice

A concern about the use of constitutive, whole-body knockout mice is the potential for unintended developmental or compensatory changes that may confound findings. In an attempt to uncover the most likely or obvious changes, studies using mAChR knockout mice have examined expression levels of the remaining mAChR subtypes, and of gene products in some other neurotransmitter systems. Surprisingly few changes were found. In $M_1^{-/-}$ mice, studies reported normal expression levels of M_2 – M_5 subtypes and dopamine receptors in all brain regions that were examined (Fisahn et al., 2002; Gerber et al., 2001; Hamilton et al., 1997; Miyakawa et al., 2001). Similarly in $M_2^{-/-}$ and $M_4^{-/-}$ mice, no changes in brain mAChR or dopamine receptor levels were reported (Gomez et al., 1999a, 1999b; Karasawa et al., 2003; Schmidt et al., 2011; Zhang et al., 2002a), and even deletion of both M_2 and M_4 subtypes did not lead to changes in M_1 or M_3 subtype levels (M_5 levels were not measured; Duttaroy et al., 2002). For the $M_5^{-/-}$ line developed at the National Institutes of Health (Wess group), no change in expression levels of M_1 – M_4 receptors, μ -opioid receptors, dopamine receptors, or dopamine transporters were detected (Basile et al., 2002; Schmidt et al., 2010; Yamada et al., 2001). However, overexpression of D_2 dopamine receptors (a key regulator of striatal dopamine release) was reported in $M_5^{-/-}$ mice developed at the University of Toronto (Yeomans group), which could account for some apparent discrepancies between findings obtained in the two strains (Wang et al., 2004). It is possible that changes in receptor levels in specific cell types or smaller brain regions went undetected when measuring levels at the brain region level. For instance, knockout of the M_2 or M_3 subtype led to measurable expression of M_1 receptors in isolated cochlea, where the receptor was not detected in wild-type mice, and $M_1^{-/-}$ mice and $M_3^{-/-}$ mice showed elevated levels of M_2 receptors in the cochlea (Maison et al., 2010). Nevertheless, it seems unlikely that compensatory changes accounted for major findings using mAChR knockout mice. As the use of tissue-specific knockout approached become more common, the development of compensatory changes may become less of a concern (see section 3, and Wess, 2012)).

2. Specific CNS functions, disease states, and drug target potential

Beyond basic research into neuronal functions, mAChR knockout mice also continue to provide insights into specific CNS functions and disease states. The medications development potential for mAChRs gained renewed attention in the past decade with the emergence of (long elusive) ligands with high subtype selectivity (Nickols and Conn, 2014). CNS conditions for which muscarinic approaches are being investigated include Alzheimer's disease, schizophrenia, substance abuse, and Parkinson's disease (reviewed in Davie et al., 2013; Dencker et al., 2012a; Foster et al., 2014a; Kruse et al., 2014; Raffa, 2009; Wess, 2012, 2004, 2003; Wess et al., 2007).

2.1. Autonomic nervous system

Muscarinic receptors mediate a wide range of functions of the parasympathetic nervous system, centrally and peripherally, and these effects were some of the first to be investigated using mAChR knockout mice. This section provides a brief overview of CNS-

related effects only. Basal body temperature is slightly decreased in $M_1^{-/-}$ mice and $M_3^{-/-}$ mice, and studies indicated that temperature regulation, either by infection or mAChR agonist-induced, is mediated through M_2 and M_3 receptors, and likely not $M_1/M_4/M_5$ receptors (Boudinot et al., 2004; Bymaster et al., 2003; Gomez et al., 1999a, 1999b; Turner et al., 2010; Yamada et al., 2001). Sleep patterns, including slow-wave and REM sleep, were recorded in $M_2^{-/-}$ mice and $M_4^{-/-}$ mice, and were normal at baseline, although $M_2^{-/-}$ mice showed altered patterns after sleep-disturbing manipulations (Turner et al., 2010). The amount of paradoxical sleep was also normal in mice lacking both M_2 and M_4 receptors, but was reduced in $M_3^{-/-}$ mice (Goutagny et al., 2005). However, analysis of theta wave electroencephalogram activity revealed altered patterns in both $M_3^{-/-}$ mice and $M_2^{-/-}M_4^{-/-}$ double knockout mice (Goutagny et al., 2005). Sleep analyses have not been reported in $M_1^{-/-}$ or $M_5^{-/-}$ mice. Finally, the ability of mAChR agonists to induce seizures appears dependent solely upon M_1 receptors, not M_2 – M_5 receptors (Bymaster et al., 2003; Hamilton et al., 1997; Takeuchi et al., 2002). M_1 receptors do not appear to play a role in induction of seizures by kainic acid or organophosphates (Hamilton et al., 1997; Kow et al., 2014; see also section 2.7 for HPA axis modulation).

2.2. Sensory systems and nociception

Deletion of one or more of the M_1 – M_4 receptors led to moderate alterations in the organization, neuronal connectivity, and/or synaptic transmission in the visual cortex, which could predict altered peripheral vision and precision of visual perception, although no gross deficits in visual acuity were detected in any strain (Groleau et al., 2014; Kuczewski et al., 2005). Indeed $M_1^{-/-}$ mice and $M_4^{-/-}$ mice performed normally in a task requiring visual discrimination of similar, complex images (Bartko et al., 2011; Bubser et al., 2014; Gould et al., 2015). M_2/M_4 , but not $M_1/M_3/M_5$ receptors, appear to play a role in cochlear function and auditory processing (Maison et al., 2010). Despite normal hearing, $M_1^{-/-}$ mice performed poorly in an auditory-cued task but normally in similar, visually-cued tasks, consistent with the abnormal cortical processing of auditory information in $M_1^{-/-}$ mice (Miyakawa et al., 2001; Zhang et al., 2006, 2005).

Stimulation of mAChRs can produce strong analgesic effects, both at the peripheral, spinal, and brain level. Analgesic effects of the non-subtype-selective mAChR agonist oxotremorine in tail flick and hot plate tests were reduced in $M_2^{-/-}$ mice, largely normal in $M_4^{-/-}$ mice, but completely lacking in mice lacking both M_2 and M_4 receptors, while effects of morphine were preserved (Duttaroy et al., 2002; Gomez et al., 1999a, 1999b). This is consistent with the functions of M_2 and M_4 receptors at the spinal level, primarily inhibiting pain transmission, while M_3 and M_5 receptors may provide subtler, more complex modulation of nociception (Chen and Pan, 2005; Chen et al., 2014, 2010; Zhang et al., 2007). Some of the analgesic effects of systemically administered mAChR agonists may also be due to stimulation of M_2 receptors on peripheral nerves (Bernardini et al., 2002; De Angelis et al., 2014). $M_1^{-/-}$ mice had normal baseline pain responses in the hot plate test but showed increased potency of morphine (Carrigan and Dykstra, 2007; Miyakawa et al., 2001). Together, those studies suggest a potential for M_2 agonists as analgesics, see also Wess et al., 2003 for review).

2.3. Cognitive function, memory, and Alzheimer's disease

As described in section 1.2, multiple mAChRs are implicated in LTP and LTD, which are essential for learning and memory functions. These functions are confirmed at the behavioral level by

specific alterations in cognitive performance in knockout mice. Pharmacological and knockout studies converge to support the role of M_1 receptors in memory consolidation, as well as in a “top down” processing (i.e., goal-oriented or rule-based processing, as opposed to “bottom-up”, sensory-driven processing) and non-matching-to-sample tasks (Anagnostaras et al., 2003; Bartko et al., 2011; Gould et al., 2015; Young and Thomas, 2014). $M_1^{-/-}$ mice, despite being generally hyperactive, show normal or improved performance in attentional function, matching-to-sample tasks, spatial reference, and reversal (Anagnostaras et al., 2003; Bartko et al., 2011; Miyakawa et al., 2001). Thus, M_1 receptors appear important for cortex-dependent processing and cortex-hippocampus interaction rather than hippocampus-dependent memory per se. $M_2^{-/-}$ mice have shown deficits in various cognitive domains including working memory, spatial learning, and behavioral flexibility, although performance sometimes normalized to wild-type level with more training (Bainbridge et al., 2008; Seeger et al., 2004; Tzavara et al., 2003). Loss of M_3 receptor signaling through a G protein-independent, phosphorylation-arrestin-dependent pathway decreased fear conditioning (Poulin et al., 2010), suggesting a use for second messenger pathway-biased allosteric M_3 receptor ligands. $M_4^{-/-}$ mice showed normal spatial reference, visual discrimination, working and episodic-like memory, but were delayed in acquiring discrimination of an interoceptive (drug) cue and showed pronounced deficits in fear conditioning (Bubser et al., 2014; Koshimizu et al., 2012; Thomsen et al., 2012; Tzavara et al., 2003).

Degeneration of cholinergic neurons projecting to cortical and hippocampal areas is central to the pathophysiology of Alzheimer's disease, the most common dementia, and M_1 agonists show promise in reducing β -amyloid plaques and neurofibrillary tangles, and in restoring cognitive function (Davie et al., 2013). In transgenic mice with amyloid precursor or tau protein mutations, used as models of Alzheimer's disease, knockout of M_1 receptors dramatically exacerbated β -amyloid and plaque formation, inflammation, tau pathology, and cognitive deficits (Davis et al., 2010; Medeiros et al., 2011). Impaired cerebrovascular function may also contribute to cognitive symptoms in Alzheimer's disease and focal cerebral ischemia. $M_5^{-/-}$ mice lack acetylcholine-induced arterial blood vessel dilation in the brain (but not peripherally), and male $M_5^{-/-}$ mice 6 months or older showed reduced cerebral blood flow, neuronal atrophy and astrocyte swelling in cortex and hippocampus, and deficits in spatial and non-spatial memory, which were corrected by estrogen (Araya et al., 2006; Kitamura et al., 2009; Yamada et al., 2001).

2.4. Schizophrenia and psychosis

Muscarinic receptor agonists have received much interest as potential targets in the treatment of schizophrenia, not only for antipsychotic actions, but also for ameliorating cognitive symptoms (Carruthers et al., 2015; Felder et al., 2001; see section 2.3). Mice lacking M_1 or M_4 receptors have phenotypes reminiscent of schizophrenia symptoms, including modest sensory gating deficits that were compounded to significant deficits in female mice lacking both receptors, as measured by prepulse inhibition (PPI) of the startle reflex (Koshimizu et al., 2012; Thomsen et al., 2010b). $M_4^{-/-}$ mice showed altered social behaviors, such as briefer contacts with other mice (Koshimizu et al., 2012). At least one line of $M_5^{-/-}$ mice also showed PPI deficits (Thomsen et al., 2007, but see Wang et al., 2004).

The moderately M_1/M_4 -selective agonist xanomeline showed some promise in clinical trials, but side effects due to off-target affinity limited its clinical usefulness. Knockout studies have

helped elucidate the mechanism of action of mAChR agonists, paving the way for more targeted approaches using selective ligands. In preclinical assays predictive of antipsychotic effects, xanomeline and the M₁/M₂/M₄ agonist BuTAC were effective in wild-type mice, M₁^{−/−} mice, and/or M₂^{−/−} mice, but effects were absent or reduced in mice lacking M₄ receptors, indicating that M₄ receptor stimulation is essential to produce antipsychotic effects (Dencker et al., 2011; Thomsen et al., 2010b; Woolley et al., 2009; see also section 3). Possible antipsychotic effects at other subtypes cannot be excluded, because the non subtype-selective agonist oxotremorine still reversed muscarinic antagonist-induced PPI deficits in mice lacking M₁, M₄, or both receptors (Thomsen et al., 2010b). Antipsychotic-like effects of typical and atypical antipsychotics (haloperidol, clozapine) were preserved in M₄^{−/−} mice as well as in M₂^{−/−}, M₅^{−/−}, M₁^{−/−}M₄^{−/−}, and M₂^{−/−}M₄^{−/−} mice, but cataleptogenic side effects were reduced in M₄^{−/−} mice (Fink-Jensen et al., 2011; Thomsen et al., 2010b, 2007; Watt et al., 2013). The involvement of non-M₄ subtypes in cataleptogenic effects have not been tested.

The above findings support the potential usefulness of mAChR ligands in the treatment of schizophrenia and psychotic disorders, in particular M₄ agonists as antipsychotics, and M₁ agonists as cognitive enhancers.

2.5. Addiction

M₁ and M₄ receptors are richly expressed in striatal tissues including the nucleus accumbens, and modulate striatal dopamine signaling, including in response to drugs of abuse. M₁^{−/−} mice, and to a lesser extent M₄^{−/−} mice, are hyperdopaminergic, hyperactive, and hypersensitive to psychomotor stimulants and dopamine agonists (Fink-Jensen et al., 2011; Gerber et al., 2001; Gomeza et al., 1999b; Guo et al., 2010a; Jeon et al., 2010; Miyakawa et al., 2001; Tzavara, 2004). Conversely, M₄^{−/−} mice have shown decreased sensitivity to dopamine antagonists (Fink-Jensen et al., 2011; Thomsen and Caine, 2016). M₄^{−/−} mice also self-administered more cocaine and alcohol than wild-type controls, although effects may extend to non-drug reinforcers (de la Cour et al., 2015; Schmidt et al., 2011). Perhaps surprisingly, M₁^{−/−} mice showed decreased conditioned place preference to cocaine and morphine, although effects on memory could explain these findings (Carrigan and Dykstra, 2007). The above findings suggest that M₁ and M₄ agonists may be of use in the treatment of drug addictions. Indeed, a combination of knockout and pharmacological approaches showed that stimulating either M₁ or M₄ receptors can attenuate effects of cocaine, including in drug self-administration (Dencker et al., 2012b; Thomsen et al., 2012, 2010a).

M₅ receptors are uniquely located in the dopaminergic reward pathway, and knockout studies revealed their role in modulating striatal dopamine (Bendor et al., 2010; Forster et al., 2002; Foster et al., 2014b; Garzón and Pickel, 2013; Wasserman et al., 2013; Yamada et al., 2001). Behavioral and molecular effects of both opioids and cocaine are attenuated in M₅^{−/−} mice (two independently generated mouse lines), including in self-administration paradigms (Basile et al., 2002; Fink-Jensen et al., 2003; Steidl et al., 2011; Steidl and Yeomans, 2009; Thomsen et al., 2005). However, at least some effects of monoamine releasers like D-amphetamine, contrary to the monoamine reuptake inhibitor cocaine, appear increased in M₅^{−/−} mice (Schmidt et al., 2010; but see Wang et al., 2004 in a different mouse line).

Taken together, the above findings suggest that M₁ and M₄ agonists, and/or M₅ antagonists, may be of use in the treatment of drug addictions. Addiction-related phenotypes in M₂^{−/−} or M₃^{−/−} mice, while not hypothesized, have not been examined directly (but see Thomsen and Caine, 2016; Joseph and Thomsen, 2017).

2.6. Parkinson's disease

Extrapyramidal control of movement is dependent upon balanced activities of muscarinic cholinergic and dopaminergic systems, loss of this balance being a hallmark of Parkinson's disease. The non subtype-selective mAChR agonist oxotremorine induces tremor, akinesia, and tremulous jaw movements that are all reversible by the anti-Parkinson treatments including L-DOPA, and are therefore used as preclinical assays in Parkinson research. Oxotremorine-induced tremor appears to be mediated entirely through M₂ receptors (i.e., normal response in mice lacking M₁, M₃, M₄, or M₅ receptors), and, remarkably, was abolished in both M₂^{−/−} mice and in heterozygous M₂^{+/−} mice (Bymaster et al., 2003; Gomeza et al., 1999a, 1999b). The cataleptogenic effects of antipsychotic drugs were attenuated in M₄^{−/−} mice, as were (moderately) oxotremorine-induced tremulous jaw movements (Fink-Jensen et al., 2011; Salamone et al., 2001; but see Karasawa et al., 2003). The incomplete loss of effect and the preserved anti-cataleptogenic effects of the mAChR antagonist scopolamine in the M₄^{−/−} mice indicates that other subtypes are involved as well (Fink-Jensen et al., 2011). Blockade of striatal M₁ and M₄ mAChRs improved motor deficits in the 6-hydroxydopamine lesion model (Ztaou et al., 2016). The usefulness of mAChR ligands may be complicated by the delicate balancing of improving motor function (mAChR antagonist approach) and cognitive function (mAChR agonist approach).

2.7. Anxiety and depression

M₄^{−/−} mice showed decreased anxiety-like behaviors in a shock-probe burying test (Degroot and Nomikos, 2006), while M₁^{−/−} mice, M₂^{−/−} mice, M₃^{−/−} mice, and M₅^{−/−} mice performed comparably to wild-type controls in the light-dark transition test and/or elevated plus-maze (Fink-Jensen et al., 2003; Miyakawa et al., 2001; Poulin et al., 2010; Seeger et al., 2004). Central, muscarinic modulation of the hypothalamic-pituitary-adrenocortical (HPA) axis, including corticosterone release, appears mediated through multiple receptors including M₁, M₃, and M₄ receptors, with some seemingly conflicting results in M₂^{−/−} mice – perhaps due to compensatory mechanisms (Gautam et al., 2009; Hemrick-Luecke et al., 2002; Rhodes et al., 2008, 2005). Muscarinic receptor antagonists may also provide a new approach to fast-acting antidepressant medication, with pharmacological and knockout studies converging on M₁ and M₂ receptors as the targets (Witkin et al., 2014).

3. Tissue-selective knockouts

Mice lacking a given mAChR selectively in the CNS (neurons and glial cells) can be generated by crossing floxed mAChR strains with *Nestin-Cre* transgenic mice. This approach uncovered surprising functions of CNS M₃ receptors: growth hormone production, as well as promotion of bone formation and inhibition of bone resorption via decreased sympathetic tone (Gautam et al., 2009; Shi et al., 2010).

Others have used the Cre-Lox approach to generate mice lacking a given receptor only in specific cell types. Selective deletion of M₄ receptors in dopamine D₁ receptor-expressing neurons (D₁-M₄^{−/−} mice) confirmed that striatal M₄ receptors are predominantly expressed in D₁-containing direct pathway MSN, as opposed to indirect pathway D₂-expressing neurons (Jeon et al., 2010). The hyperdopaminergic phenotype observed in whole-body M₄^{−/−} mice was essentially preserved in the D₁-M₄^{−/−} mice, with exaggerated response to psychomotor stimulants and D₁ receptor agonists, and blunted response to antipsychotic drugs (Jeon et al., 2010). Antipsychotic-like effects of the M₁/M₄-preferring agonist

xanomeline, and antiparkinsonian-like effects of the mAChR antagonist tropicamide, were absent in the D_1 - $M_4^{-/-}$ mice, indicating a crucial role of M_4 receptors on D_1 -expressing MSN in both effects (Dencker et al., 2011; Foster et al., 2016; Ztaou et al., 2016). In contrast, “cocaine-blocking” effects of M_4 receptor stimulation were only attenuated in the D_1 - $M_4^{-/-}$ mice, indicating that M_4 receptors on other cell types also participate in this effect (Dencker et al., 2012b). Finally, electrophysiological experiments in these mice showed that M_4 receptors located on the D_1 -expressing MSN are necessary for the development of LTD of corticostriatal glutamatergic synapses by endogenous acetylcholine signaling (Shen et al., 2015).

By a similar approach, mice lacking M_1 receptors in selected tissues have been generated: forebrain specific (FB- $M_1^{-/-}$), and further restricted to the hippocampal CA3 pyramidal neurons, or parvalbumin-containing GABAergic interneurons (PV- $M_1^{-/-}$). Unlike whole-body $M_1^{-/-}$ mice, the FB- $M_1^{-/-}$ mice and PV- $M_1^{-/-}$ mice were not hyperactive, in agreement with the notion that the hyperactivity stems from effects in striatal tissues (Kamsler et al., 2010; Yi et al., 2014). CA3 M_1 receptors were found to be necessary for presynaptic induction of glutamatergic LTD, while PV M_1 receptors appear to play a role in PV interneuron excitability, learning/memory and mAChR agonist-induced seizures (Kamsler et al., 2010; Yi et al., 2014).

4. Conclusions

Knockout mice have proved an invaluable tool in elucidating physiological functions of CNS mAChRs. Studies using knockout mice helped demonstrate how acetylcholine from local interneurons and projection neurons provides powerful and complex modulation of neuronal activity and plasticity via different mAChR subtypes, in brain regions such as the striatum, hippocampus, and neocortex. Phenotypic analysis of mAChR knockout mice revealed deficits or changes in CNS functions ranging from basic autonomic functions like body temperature regulation and sleep, over motor and sensory systems, to complex cognitive functions. Combined with recently developed allosteric subtype-selective agonists, antagonists, and modulators, knockout mouse experiments have uncovered promising new medications development possibilities, which indicate that mAChR ligands may be of use in the treatment of pain, Alzheimer's disease and other memory/cognitive impairment, schizophrenia, addiction, depression, and more.

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References

- Anagnostaras, S.G., Murphy, G.G., Hamilton, S.E., Mitchell, S.L., Rahnema, N.P., Nathanson, N.M., Silva, A.J., 2003. Selective cognitive dysfunction in acetylcholine M_1 muscarinic receptor mutant mice. *Nat. Neurosci.* 6, 51–58.
- Anisuzzaman, A.S.M., Uwada, J., Masuoka, T., Yoshiki, H., Nishio, M., Ikegaya, Y., Takahashi, N., Matsuki, N., Fujibayashi, Y., Yonekura, Y., Momiyama, T., Muramatsu, I., 2013. Novel contribution of cell surface and intracellular M_1 -muscarinic acetylcholine receptors to synaptic plasticity in hippocampus. *J. Neurochem.* 126, 360–371.
- Araya, R., Noguchi, T., Yuhki, M., Kitamura, N., Higuchi, M., Saido, T.C., Seki, K., Itoharu, S., Kawano, M., Tanemura, K., Takashima, A., Yamada, K., Kondoh, Y., Kanno, I., Wess, J., Yamada, M., 2006. Loss of M_5 muscarinic acetylcholine receptors leads to cerebrovascular and neuronal abnormalities and cognitive deficits in mice. *Neurobiol. Dis.* 24, 334–344.

- Bainbridge, N.K., Koselke, L.R., Jeon, J., Bailey, K.R., Wess, J., Crawley, J.N., Wrenn, C.C., 2008. Learning and memory impairments in a congenic C57BL/6 strain of mice that lacks the M_2 muscarinic acetylcholine receptor subtype. *Behav. Brain Res.* 190, 50–58.
- Bartko, S.J., Romberg, C., White, B., Wess, J., Bussey, T.J., Saksida, L.M., 2011. Intact attentional processing but abnormal responding in M_1 muscarinic receptor-deficient mice using an automated touchscreen method. *Neuropharmacology* 61, 1366–1378.
- Basile, A.S., Fedorova, I., Zapata, A., Liu, X., Shippenberg, T., Duttaroy, A., Yamada, M., Wess, J., 2002. Deletion of the M_5 muscarinic acetylcholine receptor attenuates morphine reinforcement and withdrawal but not morphine analgesia. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11452–11457.
- Bendor, J., Lizardi-Ortiz, J.E., Westphalen, R.L., Brandstetter, M., Hemmings, H.C., Sulzer, D., Flajolet, M., Greengard, P., 2010. AGAP1/AP-3-dependent endocytic recycling of M_5 muscarinic receptors promotes dopamine release. *EMBO J.* 29, 2813–2826.
- Berkeley, J.L., Gomez, J., Wess, J., Hamilton, S.E., Nathanson, N.M., Levey, A.I., 2001. M_1 muscarinic acetylcholine receptors activate extracellular signal-regulated kinase in CA1 pyramidal neurons in mouse hippocampal slices. *Mol. Cell. Neurosci.* 18, 512–524.
- Bernard, V., Normand, E., Bloch, B., 1992. Phenotypic characterization of the rat striatal neurons expressing muscarinic receptor genes. *J. Neurosci.* 12, 3591–3600.
- Bernardini, N., Roza, C., Sauer, S.K., Gomez, J., Wess, J., Reeh, P.W., 2002. Muscarinic M_2 receptors on peripheral nerve endings: a molecular target of antinociception. *J. Neurosci.* 22, RC229. doi:20026476.
- Bonsi, P., Martella, G., Cuomo, D., Platania, P., Sciamanna, G., Bernardi, G., Wess, J., Pisani, A., 2008. Loss of muscarinic autoreceptor function impairs long-term depression but not long-term potentiation in the striatum. *J. Neurosci.* 28, 6258–6263.
- Boudinot, E., Yamada, M., Wess, J., Champagnat, J., Foutz, A.S., 2004. Ventilatory pattern and chemosensitivity in M_1 and M_3 muscarinic receptor knockout mice. *Respir. Physiol. Neurobiol.* 139, 237–245.
- Bubser, M., Bridges, T.M., Dencker, D., Gould, R.W., Grannan, M., Noetzel, M.J., Lamsal, A., Niswender, C.M., Daniels, J.S., Poslusney, M.S., Melancon, B.J., Tarr, J.C., Byers, F.W., Wess, J., Duggan, M.E., Dunlop, J., Wood, M.W., Brandon, N.J., Wood, M.R., Lindsley, C.W., Conn, P.J., Jones, C.K., 2014. Selective activation of M_4 muscarinic acetylcholine receptors reverses MK-801-induced behavioral impairments and enhances associative learning in rodents. *ACS Chem. Neurosci.* 5, 920–942.
- Bymaster, F.P., Carter, P.A., Yamada, M., Gomez, J., Wess, J., Hamilton, S.E., Nathanson, N.M., McKinzie, D.L., Felder, C.C., 2003. Role of specific muscarinic receptor subtypes in cholinergic parasympathomimetic responses, in vivo phosphoinositide hydrolysis, and pilocarpine-induced seizure activity. *Eur. J. Neurosci.* 17, 1403–1410.
- Carrigan, K.A., Dykstra, L.A., 2007. Behavioral effects of morphine and cocaine in M_1 muscarinic acetylcholine receptor-deficient mice. *Psychopharmacol. Berl.* 191, 985–993.
- Carruthers, S.P., Gurvich, C.T., Rossell, S.L., 2015. The muscarinic system, cognition and schizophrenia. *Neurosci. Biobehav. Rev.* 55, 393–402.
- Cea-del Rio, C.A., Lawrence, J.J., Tricoire, L., Erdelyi, F., Szabo, G., McBain, C.J., 2010. M_3 muscarinic acetylcholine receptor expression confers differential cholinergic modulation to neurochemically distinct hippocampal basket cell subtypes. *J. Neurosci.* 30, 6011–6024.
- Cea-del Rio, C.A., Lawrence, J.J., Erdelyi, F., Szabo, G., McBain, C.J., 2011. Cholinergic modulation amplifies the intrinsic oscillatory properties of CA1 hippocampal cholecystinin-positive interneurons. *J. Physiol.* 589, 609–627.
- Chen, S., Pan, H., 2005. Functional activity of the M_2 and M_4 receptor subtypes in the spinal cord studied with muscarinic acetylcholine receptor knockout mice. *J. Pharmacol. Exp. Ther.* 313, 765–770.
- Chen, S.R., Chen, H., Yuan, W.X., Wess, J., Pan, H.L., 2014. Differential regulation of primary afferent input to spinal cord by muscarinic receptor subtypes delineated using knockout mice. *J. Biol. Chem.* 289, 14321–14330.
- Chen, S.R., Chen, H., Yuan, W.X., Wess, J., Pan, H.L., 2010. Dynamic control of glutamatergic synaptic input in the spinal cord by muscarinic receptor subtypes defined using knockout mice. *J. Biol. Chem.* 285, 40427–40437.
- Dasari, S., Gullledge, A.T., 2011. M_1 and M_4 receptors modulate hippocampal pyramidal neurons. *J. Neurophysiol.* 105, 779–792.
- Davie, B.J., Christopoulos, A., Scammells, P.J., 2013. Development of M_1 mAChR allosteric and bitopic ligands: prospective therapeutics for the treatment of cognitive deficits. *ACS Chem. Neurosci.* 4, 1026–1048.
- Davis, A.A., Fritz, J.J., Wess, J., Lah, J.J., Levey, A.I., 2010. Deletion of M_1 muscarinic acetylcholine receptors increases amyloid pathology in vitro and in vivo. *J. Neurosci.* 30, 4190–4196.
- de la Cour, C., Sørensen, G., Wortwein, G., Weikop, P., Dencker, D., Fink-Jensen, A., Molander, A., 2015. Enhanced self-administration of alcohol in muscarinic acetylcholine M_4 receptor knockout mice. *Eur. J. Pharmacol.* 746, 1–5.
- Degroote, A., Nomikos, G.G., 2006. Genetic deletion of muscarinic M_4 receptors is anxiolytic in the shock-probe burying model. *Eur. J. Pharmacol.* 531, 183–186.
- Dencker, D., Thomsen, M., Wortwein, G., Weikop, P., Cui, Y., Jeon, J., Wess, J., Fink-Jensen, A., 2012a. Muscarinic acetylcholine receptor subtypes as potential drug targets for the treatment of schizophrenia, drug abuse, and Parkinson's disease. *ACS Chem. Neurosci.* 3, 80–89.
- Dencker, D., Weikop, P., Sørensen, G., Woldbye, D.P.D., Wortwein, G., Wess, J., Fink-Jensen, A., 2012b. An allosteric enhancer of M_4 muscarinic acetylcholine

- receptor function inhibits behavioral and neurochemical effects of cocaine. *Psychopharmacol. Berl.* 224, 277–287.
- Dencker, D., Wörtwein, G., Weikop, P., Jeon, J., Thomsen, M., Sager, T.N., Mørk, A., Woldbye, D.P.D., Wess, J., Fink-Jensen, A., 2011. Involvement of a subpopulation of neuronal M4 muscarinic acetylcholine receptors in the antipsychotic-like effects of the M1/M4 preferring muscarinic receptor agonist xanomeline. *J. Neurosci.* 31, 5905–5908.
- Dennis, S.H., Pasqui, F., Colvin, E.M., Sanger, H., Mogg, A.J., Felder, C.C., Broad, L.M., Fitzjohn, S.M., Isaac, J.T.R., Mellor, J.R., 2016. Activation of muscarinic M1 acetylcholine receptors induces long-term potentiation in the Hippocampus. *Cereb. Cortex* 26, 414–426.
- De Angelis, F., Marinelli, S., Fioretti, B., Catacuzzeno, L., Franciolini, F., Pavone, F., Tata, A.M., 2014. M2 receptors exert analgesic action on DRG sensory neurons by negatively modulating VR1 activity. *J. Cell. Physiol.* 229, 783–790.
- Ding, J.B., Guzman, J.N., Peterson, J.D., Goldberg, J.A., Surmeier, D.J., 2010. Thalamic gating of corticostriatal signaling by cholinergic interneurons. *Neuron* 67, 294–307.
- Duttaroy, A., Gomez, J., Gan, J.-W., Siddiqui, N., Basile, A.S., Harman, W.D., Smith, P.L., Felder, C.C., Levey, A.I., Wess, J., 2002. Evaluation of muscarinic agonist-induced analgesia in muscarinic acetylcholine receptor knockout mice. *Mol. Pharmacol.* 62, 1084–1093.
- Felder, C., Porter, A.C., Skillman, T.L., Zhang, L., Bymaster, F.P., Nathanson, N.M., Hamilton, S.E., Gomez, J., Wess, J., McKinzie, D.L., 2001. Elucidating the role of muscarinic receptors in psychosis. *Life Sci.* 68, 2605–2613.
- Fink-Jensen, A., Fedorova, I., Wortwein, G., Woldbye, D.P.D., Rasmussen, T., Thomsen, M., Bolwig, T.G., Knitowski, K.M., McKinzie, D.L., Yamada, M., Basile, A., 2003. Role for M5 Muscarinic Acetylcholine Receptors in Cocaine Addiction, vol.96, pp. 91–96.
- Fink-Jensen, A., Schmidt, L.S., Dencker, D., Schüle, C., Wess, J., Wortwein, G., Woldbye, D.P.D., 2011. Antipsychotic-induced Catalepsy Is Attenuated in Mice Lacking the M4 Muscarinic Acetylcholine Receptor, vol.656, pp. 39–44.
- Fisahn, A., Yamada, M., Duttaroy, A., Gan, J., Deng, C., McBain, C.J., Wess, J., 2002. Muscarinic induction of hippocampal gamma oscillations requires coupling of the M1. *Neuron* 33, 615–624.
- Forster, G.L., Yeomans, J.S., Takeuchi, J., Blaha, C.D., 2002. M5 muscarinic receptors are required for prolonged accumbal dopamine release after electrical stimulation of the pons in mice. *J. Neurosci.* 22, RC190. doi:20015912.
- Foster, D.J., Choi, D.L., Conn, P.J., Rook, J.M., 2014a. Activation of M1 and M4 muscarinic receptors as potential treatments for Alzheimer's disease and schizophrenia. *Neuropsychiatr. Dis. Treat.* 10, 183–191.
- Foster, D.J., Gentry, P.R., Lizardi-Ortiz, J.E., Bridges, T.M., Wood, M.R., Niswender, C.M., Sulzer, D., Lindsley, C.W., Xiang, Z., Conn, P.J., 2014b. M5 receptor activation produces opposing physiological outcomes in dopamine neurons depending on the receptor's location. *J. Neurosci.* 34, 3253–3262.
- Foster, D.J., Wilson, J.M., Remke, D.H., Mahmood, M.S., Uddin, M.J., Wess, J., Patel, S., Marnett, L.J., Niswender, C.M., Jones, C.K., Xiang, Z., Lindsley, C.W., Rook, J.M., Conn, P.J., 2016. Antipsychotic-like effects of M4 positive allosteric modulators are mediated by CB2 receptor-dependent inhibition of dopamine release. *Neuron* 91, 1–9.
- Fukudome, Y., Ohno-Shosaku, T., Matsui, M., Omori, Y., Fukaya, M., Tsubokawa, H., Taketo, M.M., Watanabe, M., Manabe, T., Kano, M., Ohno-Shosaku, A.T., Matsui, A.M., 2004. Two distinct classes of muscarinic action on hippocampal inhibitory synapses: M2-mediated direct suppression and M1/M3-mediated indirect suppression through endocannabinoid signalling. *Eur. J. Neurosci.* 19, 2682–2692.
- Garzón, M., Pickel, V.M., 2013. Somatodendritic targeting of M5 muscarinic receptor in the rat ventral tegmental area: implications for mesolimbic dopamine transmission. *J. Comp. Neurol.* 521, 2927–2946.
- Gautam, D., Duttaroy, A., Cui, Y., Han, S.-J., Deng, C., Seeger, T., Alzheimer, C., Wess, J., 2006. M1–M3 muscarinic acetylcholine receptor-deficient mice. *J. Mol. Neurosci.* 30, 157–160.
- Gautam, D., Jeon, J., Starost, M.F., Han, S.-J., Hamdan, F.F., Cui, Y., Parlow, A.F., Gavrilova, O., Szalayova, I., Mezey, E., Wess, J., 2009. Neuronal M3 muscarinic acetylcholine receptors are essential for somatotroph proliferation and normal somatic growth. *Proc. Natl. Acad. Sci. U. S. A.* 106, 6398–6403.
- Gerber, D.J., Sotnikova, T.D., Gainetdinov, R.R., Huang, S.Y., Caron, M.G., Tonegawa, S., 2001. Hyperactivity, elevated dopaminergic transmission, and response to amphetamine in M1 muscarinic acetylcholine receptor-deficient mice. *Proc. Natl. Acad. Sci. U. S. A.* 98, 15312–15317.
- Goldberg, J.A., Ding, J.B., Surmeier, D.J., 2012. Muscarinic modulation of striatal function and circuitry. *Handb. Exp. Pharmacol.* 208, 223–241.
- Gomez, J., Shannon, H., Kostenis, E., Felder, C., Zhang, L., Brodtkin, J., Grinberg, A., Sheng, H., Wess, J., 1999a. Pronounced pharmacologic deficits in M2 muscarinic acetylcholine receptor knockout mice. *Proc. Natl. Acad. Sci. U. S. A.* 96, 1692–1697.
- Gomez, J., Zhang, L., Kostenis, E., Felder, C., Bymaster, F., Brodtkin, J., Shannon, H., Xia, B., Deng, C., Wess, J., 1999b. Enhancement of D1 dopamine receptor-mediated locomotor stimulation in M(4) muscarinic acetylcholine receptor knockout mice. *Proc. Natl. Acad. Sci. U. S. A.* 96, 10483–10488.
- Gonzales, K.K., Smith, Y., 2015. Cholinergic interneurons in the dorsal and ventral striatum: anatomical and functional considerations in normal and diseased conditions. *Ann. N. Y. Acad. Sci.* 1349, 1–45.
- Gould, R.W., Dencker, D., Grannan, M., Busber, M., Zhan, X., Wess, J., Xiang, Z., Lucason, C., Lindsley, C.W., Conn, P.J., Jones, C.K., 2015. Role for the M1 muscarinic acetylcholine receptor in top-down cognitive processing using a touchscreen visual discrimination task in mice. *ACS Chem. Neurosci.* 6, 1683–1695.
- Goutagny, R., Comte, J.-C., Salvetti, D., Gomez, J., Yamada, M., Wess, J., Luppi, P.-H., Fort, P., 2005. Paradoxical sleep in mice lacking M3 and M2/M4 muscarinic receptors. *Neuropsychobiology* 52, 140–146.
- Groleau, M., Nguyen, H.N., Vanni, M.P., Huppé-Gourguet, F., Casanova, C., Vaucher, E., 2014. Impaired functional organization in the visual cortex of muscarinic receptor knock-out mice. *Neuroimage* 98, 233–242.
- Gulledge, A.T., Bucci, D.J., Zhang, S.S., Matsui, M., Yeh, H.H., 2009. M1 receptors mediate cholinergic modulation of excitability in neocortical pyramidal neurons. *J. Neurosci.* 29, 9888–9902.
- Guo, J., Hazra, R., Dabrowska, J., Muly, E.C., J.W., G.R.D., 2012. Presynaptic muscarinic M2 receptors modulate glutamatergic transmission in the bed nucleus of the stria terminalis. *Neuropharmacology* 62, 1671–1683.
- Guo, M.-L., Fibich, E.E., Liu, X.-Y., Choe, E.S., Buch, S., Mao, L.-M., Wang, J.Q., 2010a. CaMKIIα interacts with M4 muscarinic receptors to control receptor and psychomotor function. *EMBO J.* 29, 2070–2081.
- Guo, M.-L., Liu, Z., Chu, X.-P., Mao, L.-M., Wang, J.Q., 2010b. CaMKIIα, a modulator of M4 muscarinic acetylcholine receptors. *Commun. Integr. Biol.* 3, 465–467.
- Hamilton, S.E., Loose, M.D., Qi, M., Levey, A.I., Hille, B., McKnight, G.S., Idzerda, R.L., Nathanson, N.M., 1997. Disruption of the m1 receptor gene ablates muscarinic receptor-dependent M current regulation and seizure activity in mice. *Proc. Natl. Acad. Sci. U. S. A.* 94, 13311–13316.
- Hamilton, S.E., Nathanson, N.M., 2001. The M1 receptor is required for muscarinic activation of mitogen-activated protein (MAP) kinase in murine cerebral cortical neurons. *J. Biol. Chem.* 276, 15850–15853.
- Hemrick-Luecke, S.K., Bymaster, F.P., Evans, D.C., Wess, J., Felder, C.C., 2002. Muscarinic agonist-mediated increases in serum corticosterone levels are abolished in m(2) muscarinic acetylcholine receptor knockout mice. *J. Pharmacol. Exp. Ther.* 303, 99–103.
- Higley, M.J., Soler-Llavina, G.J., Sabatini, B.L., 2009. Cholinergic modulation of multivesicular release regulates striatal synaptic potency and integration. *Nat. Neurosci.* 12, 1121–1128.
- Ince, E., Ciliax, B.J., Levey, A.I., 1997. Differential expression of D1 and D2 dopamine and m4 muscarinic acetylcholine receptor proteins in identified striatonigral neurons. *Synapse* 27, 357–366.
- Jeon, J., Dencker, D., Wörtwein, G., Woldbye, D.P.D., Cui, Y., Davis, A.A., Levey, A.I., Schütz, G., Sager, T.N., Mørk, A., Li, C., Deng, C.-X., Fink-Jensen, A., Wess, J., 2010. A subpopulation of neuronal M4 muscarinic acetylcholine receptors plays a critical role in modulating dopamine-dependent behaviors. *J. Neurosci.* 30, 2396–2405.
- Joseph, L., Thomsen, M., 2017. Effects of muscarinic receptor antagonists on cocaine discrimination in wild-type mice and in muscarinic receptor M1, M2, and M4 receptor knockout mice. *Behav. Brain Res.* 329, 75–83.
- Kamsler, A., McHugh, T.J., Gerber, D., Huang, S.Y., Tonegawa, S., 2010. Presynaptic M1 muscarinic receptors are necessary for mGluR long-term depression in the hippocampus. *Proc. Natl. Acad. Sci. U. S. A.* 107, 1618–1623.
- Karasawa, H., Taketo, M.M., Matsui, M., 2003. Loss of anti-cataleptic effect of scopolamine in mice lacking muscarinic acetylcholine receptor subtype 4. *Eur. J. Pharmacol.* 468, 15–19.
- Kitamura, N., Araya, R., Kudoh, M., Kishida, H., Kimura, T., Murayama, M., Takashima, A., Sakamaki, Y., Hashikawa, T., Ito, S., Ohtsuki, S., Terasaki, T., Wess, J., Yamada, M., 2009. Beneficial effects of estrogen in a mouse model of cerebrovascular insufficiency. *PLoS One* 4.
- Kohlmeier, K.A., Ishibashi, M., Wess, J., Bickford, M.E., Leonard, C.S., 2012. Knockouts reveal overlapping functions of M2 and M4 muscarinic receptors and evidence for a local glutamatergic circuit in the laterodorsal tegmental nucleus. *J. Neurophysiol.* 108, 2751–2766.
- Koshimizu, H., Leiter, L.M., Miyakawa, T., 2012. M4 muscarinic receptor knockout mice display abnormal social behavior and decreased prepulse inhibition. *Mol. Brain* 5, 10.
- Kow, R.L., Jiang, K., Naydenov, A.V., Le, J.H., Stella, N., Nathanson, N.M., 2014. Modulation of pilocarpine-induced seizures by cannabinoid receptor 1. *PLoS One* 9, 1–8.
- Kremin, T., Gerber, D., Giocomo, L.M., Huang, S.Y., Tonegawa, S., Hasselmo, M.E., 2006. Muscarinic suppression in stratum radiatum of CA1 shows dependence on presynaptic M1 receptors and is not dependent on effects at GABAB receptors. *Neurobiol. Learn. Mem.* 85, 153–163.
- Kruse, A.C., Kobilka, B.K., Gautam, D., Sexton, P.M., Christopoulos, A., Wess, J., 2014. Muscarinic acetylcholine receptors: novel opportunities for drug development. *Nat. Rev. Drug Discov.* 13, 549–560.
- Kuczewski, N., Aztiria, E., Gautam, D., Wess, J., Domenici, L., 2005. Acetylcholine modulates cortical synaptic transmission via different muscarinic receptors, as studied with receptor knockout mice. *J. Physiol.* 566, 907–919.
- Maison, S.F., Liu, X.-P., Vetter, D.E., Eatock, R.A., Nathanson, N.M., Wess, J., Liberman, M.C., 2010. Muscarinic signaling in the cochlea: presynaptic and postsynaptic effects on efferent feedback and afferent excitability. *J. Neurosci.* 30, 6751–6762.
- Martella, G., Tassone, A., Sciamanna, G., Platania, P., Cuomo, D., Viscomi, M.T., Bonsi, P., Cacci, E., Biagioni, S., Usiello, A., Bernardi, G., Sharma, N., Standera, D.G., Pisani, A., 2009. Impairment of bidirectional synaptic plasticity in the striatum of a mouse model of DYT1 dystonia: role of endogenous acetylcholine. *Brain* 132, 2336–2349.
- Matsui, M., Yamada, S., Oki, T., Manabe, T., Taketo, M.M., Ehler, F.J., 2004. Functional analysis of muscarinic acetylcholine receptors using knockout mice. *Life Sci.* 75,

- 2971–2981.
- Medeiros, R., Kitazawa, M., Caccamo, A., Baglietto-Vargas, D., Estrada-Hernandez, T., Cribbs, D.H., Fisher, A., Laferla, F.M., 2011. Loss of muscarinic M1 receptor exacerbates Alzheimer's disease-like pathology and cognitive decline. *Am. J. Pathol.* 179, 980–991.
- Miyakawa, T., Yamada, M., Duttaroy, A., Wess, J., 2001. Hyperactivity and intact hippocampus-dependent learning in mice lacking the M1 muscarinic acetylcholine receptor. *J. Neurosci.* 21, 5239–5250.
- Narushima, M., Uchigashima, M., Fukaya, M., Matsui, M., Manabe, T., Hashimoto, K., Watanabe, M., Kano, M., 2007. Tonic enhancement of endocannabinoid-mediated retrograde suppression of inhibition by cholinergic interneuron activity in the striatum. *J. Neurosci.* 27, 496–506.
- Nickols, H.H., Conn, J.P., 2014. Development of allosteric modulators of GPCRs for treatment of CNS disorders. *Neurobiol. Dis.* 61, 55–71.
- Ohno-Shosaku, T., Matsui, M., Fukudome, Y., Shosaku, J., Tsubokawa, H., Taketo, M.M., Manabe, T., Kano, M., 2003. Postsynaptic M1 and M3 receptors are responsible for the muscarinic enhancement of retrograde endocannabinoid signalling in the hippocampus. *Eur. J. Neurosci.* 18, 109–116.
- Onali, P., Olanas, M.C., 2002. Muscarinic M4 receptor inhibition of dopamine D1-like receptor signalling in rat nucleus accumbens. *Eur. J. Pharmacol.* 448, 105–111.
- Origlia, N., Kuczewski, N., Aztiria, E., Gautam, D., Wess, J., Domenici, L., 2006. Muscarinic acetylcholine receptor knockout mice show distinct synaptic plasticity impairments in the visual cortex. *J. Physiol.* 577, 829–840.
- Pakhotin, P., Bracci, E., 2007. Cholinergic interneurons control the excitatory input to the striatum. *J. Neurosci.* 27, 391–400.
- Pancani, T., Bolarinwa, C., Smith, Y., Lindsley, C., Conn, P., Xiang, Z., 2014. M4 machr-mediated modulation of glutamatergic transmission at corticostriatal synapses. *ACS Chem. Neurosci.* 5, 318–324.
- Piccio, M.R., Higley, M.J., Mineur, Y.S., 2012. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. *Neuron* 76, 116–129.
- Poulin, B., Butcher, A., McWilliams, P., Bourgognon, J., Pawlak, R., Choi, K., 2010. The M3–muscarinic receptor regulates learning and memory in a receptor phosphorylation/arrestin-dependent manner. *Proc. Natl. Acad. Sci. U. S. A.* 107, 9440–9445.
- Raffa, R.B., 2009. The M5 muscarinic receptor as possible target for treatment of drug abuse. *J. Clin. Pharm. Ther.* 34, 623–629.
- Rhodes, M.E., Billings, T.E., Czambel, R.K., Rubin, R.T., 2005. Pituitary-adrenal responses to cholinergic stimulation and acute mild stress are differentially elevated in male and female M2 muscarinic receptor knockout mice. *J. Neuroendocrinol.* 17, 817–826.
- Rhodes, M.E., Rubin, R.T., McKlveen, J.M., Karwowski, T.E., Fulton, B.A., Czambel, R.K., 2008. Pituitary–Adrenal responses to oxotremorine and acute stress in male and female M₁ muscarinic receptor knockout mice: comparisons to M₂ muscarinic receptor knockout mice. *J. Neuroendocrinol.* 20, 617–625.
- Rinaldo, L., Hansel, C., 2013. Muscarinic acetylcholine receptor activation blocks long-term potentiation at cerebellar parallel fiber–Purkinje cell synapses via cannabinoid signaling. *Proc. Natl. Acad. Sci. U. S. A.* 110, 11181–11186.
- Rouse, S.T., Hamilton, S.E., Potter, L.T., Nathanson, N.M., Conn, P.J., 2000. Muscarinic-induced modulation of potassium conductances is unchanged in mouse hippocampal pyramidal cells that lack functional M1 receptors. *Neurosci. Lett.* 278, 61–64.
- Salamone, J.D., Correa, M., Carlson, B.B., Wisniecki, A., Mayorga, A.J., Nisenbaum, E., Nisenbaum, L., Felder, C., 2001. Neostriatal muscarinic receptor subtypes involved in the generation of tremulous jaw movements in rodents: implications for cholinergic involvement in Parkinsonism. *Life Sci.* 68, 2579–2584.
- Schmidt, L.S., Miller, A.D., Lester, D.B., Bay-Richter, C., Schüle, C., Frikke-Schmidt, H., Wess, J., Blaha, C.D., Woldbye, D.P.D., Fink-Jensen, A., Wortwein, G., Schmidt, H.F., 2010. Increased amphetamine-induced locomotor activity, sensitization, and accumbal dopamine release in M5 muscarinic receptor knockout mice. *Psychopharmacol. Berl.* 207, 547–558.
- Schmidt, L.S., Thomsen, M., Weikop, P., Dencker, D., Wess, J., Woldbye, D.P.D., Wortwein, G., Fink-Jensen, A., 2011. Increased cocaine self-administration in M4 muscarinic acetylcholine receptor knockout mice. *Psychopharmacol. Berl.* 216, 367–378.
- Seeger, T., Fedorova, I., Zheng, F., Miyakawa, T., Koustova, E., Gomez, J., Basile, A.S., Alzheimer, C., Wess, J., 2004. M-2 muscarinic acetylcholine receptor knock-out mice show deficits in behavioral flexibility, working memory, and hippocampal plasticity. *J. Neurosci.* 24, 10117–10127.
- Shen, W., 2005. Cholinergic suppression of KCNQ channel currents enhances excitability of striatal medium spiny neurons. *J. Neurosci.* 25, 7449–7458.
- Shen, W., Plotkin, J.L., Francardo, V., Ko, W.K.D., Xie, Z., Li, Q., Fieblinger, T., Wess, J., Neubig, R.R., Lindsley, C.W., Conn, P.J., Greengard, P., Bezard, E., Cenci, M.A., Surmeier, D.J., 2015. M4 muscarinic receptor signaling ameliorates striatal plasticity deficits in models of L-DOPA-induced dyskinesia. *Neuron* 88, 762–773.
- Shi, Y., Oury, F., Yadav, V.K., Wess, J., Liu, X.S., Guo, X.E., Murshed, M., Karsenty, G., 2010. Signaling through the M3 muscarinic receptor favors bone mass accrual by decreasing sympathetic activity. *Cell Metab.* 11, 231–238.
- Shin, J.H., Adrover, M.F., Wess, J., Alvarez, V.A., 2015. Muscarinic regulation of dopamine and glutamate transmission in the nucleus accumbens. *Proc. Natl. Acad. Sci.* 12, 8124–8129.
- Shinoe, T., 2005. Modulation of synaptic plasticity by physiological activation of M1 muscarinic acetylcholine receptors in the mouse Hippocampus. *J. Neurosci.* 25, 11194–11200.
- Shirey, J.K., Xiang, Z., Orton, D., Brady, A.E., Johnson, K.A., Williams, R., Weaver, D., Niswender, C.M., Ayala, J.E., Rodriguez, A.L., Conn, P.J., 2008. An Allosteric Potentiator of M4 MACHR Modulates Hippocampal Synaptic Transmission, vol. 4, pp. 42–50.
- Smythies, J., 2005. Section I. The cholinergic system. *Int. Rev. Neurobiol.* 64, 1–122.
- Steidl, S., Miller, A.D., Blaha, C.D., Yeomans, J.S., 2011. M5 muscarinic receptors mediate striatal dopamine activation by ventral tegmental morphine and pedunculopontine stimulation in mice. *PLoS One* 6.
- Steidl, S., Yeomans, J.S., 2009. M5 muscarinic receptor knockout mice show reduced morphine-induced locomotion but increased locomotion after cholinergic antagonism in the ventral tegmental area. *J. Pharmacol. Exp. Ther.* 328, 263–275.
- Takeuchi, J., Fulton, J., Jia, Z., ping, Abramov-Newerly, W., Jamot, L., Sud, M., Coward, D., Ralph, M., Roder, J., Yeomans, J., 2002. Increased drinking in mutant mice with truncated M5 muscarinic receptor genes. *Pharmacol. Biochem. Behav.* 72, 117–123.
- Thomsen, M., Caine, S.B., 2016. Effects of dopamine D1-like and D2-like antagonists on cocaine discrimination in muscarinic receptor knockout mice. *Eur. J. Pharmacol.* 776, 71–80.
- Thomsen, M., Conn, P.J., Lindsley, C., Boon, J.Y., Fulton, B.S., Fink-jensen, A., Caine, S.B., Wess, J., Boon, J.Y., Fulton, B.S., Fink-jensen, A., Caine, S.B., 2010a. Attenuation of cocaine's reinforcing and discriminative stimulus effects via muscarinic M1 acetylcholine receptor stimulation. *J. Pharmacol. Exp. Ther.* 332, 959–969.
- Thomsen, M., Lindsley, C.W., Conn, P.J., Wessell, J.E., Fulton, B.S., Wess, J., Caine, S.B., 2012. Contribution of both M1 and M4 receptors to muscarinic agonist-mediated attenuation of the cocaine discriminative stimulus in mice. *Psychopharmacol. Berl.* 220, 673–685.
- Thomsen, M., Wess, J., Fulton, B.S., Fink-Jensen, A., Caine, S.B., 2010b. Modulation of prepulse inhibition through both M1 and M4 muscarinic receptors in mice. *Psychopharmacol. Berl.* 208, 401–416.
- Thomsen, M., Woldbye, D.P.D., Wortwein, G., Fink-Jensen, A., Wess, J., Caine, S.B., 2005. Reduced cocaine self-administration in muscarinic M5 acetylcholine receptor-deficient mice. *J. Neurosci.* 25, 8141–8149.
- Thomsen, M., Wortwein, G., Fink-Jensen, A., Woldbye, D.P.D., Wess, J., Caine, S.B., 2007. Decreased prepulse inhibition and increased sensitivity to muscarinic, but not dopaminergic drugs in M5 muscarinic acetylcholine receptor knockout mice. *Psychopharmacol. Berl.* 192, 97–110.
- Threlfell, S., Clements, M.A., Khodai, T., Pienaar, I.S., Exley, R., Wess, J., Cragg, S.J., 2010. Striatal muscarinic receptors promote activity dependence of dopamine transmission via distinct receptor subtypes on cholinergic interneurons in ventral versus dorsal striatum. *J. Neurosci.* 30, 3398–3408.
- Threlfell, S., Cragg, S.J., 2011. Dopamine signaling in dorsal versus ventral striatum: the dynamic role of cholinergic interneurons. *Front. Syst. Neurosci.* 5, 11.
- Turner, J., Hughes, L.F., Toth, L.A., 2010. Sleep, activity, temperature and arousal responses of mice deficient for muscarinic receptor M2 or M4. *Life Sci.* 86, 158–169.
- Tzavara, E.T., 2004. M4 muscarinic receptors regulate the dynamics of cholinergic and dopaminergic neurotransmission: relevance to the pathophysiology and treatment of related central nervous system pathologies. *FASEB J.* 18, 1–18.
- Tzavara, E.T., Bymaster, F.P., Felder, C.C., Wade, M., Gomez, J., Wess, J., McKinzie, D.L., Nomikos, G.G., 2003. Dysregulated hippocampal acetylcholine neurotransmission and impaired cognition in M2, M4 and M2/M4 muscarinic receptor knockout mice. *Mol. Psychiatry* 8, 673–679.
- Tzavara, E.T., Bymaster, F.P., Nomikos, G.G., 2006. The procholinergic effects of the atypical antipsychotic olanzapine are independent of muscarinic autoreceptor inhibition. *Mol. Psychiatry* 11, 619–621.
- Vilaro, M.T., Palacios, J.M., Mengod, G., 1990. Localization of m5 muscarinic receptor mRNA in rat brain examined by in situ hybridization histochemistry. *Neurosci. Lett.* 114, 154–159.
- Wang, H., Ng, K., Hayes, D., Gao, X., Forster, G., Blaha, C., Yeomans, J., 2004. Decreased amphetamine-induced locomotion and improved latent inhibition in mice mutant for the M5 muscarinic receptor gene found in the human 15q schizophrenia region. *Neuropsychopharmacology* 29, 2126–2139.
- Wasserman, D.I., Wang, H.G., Rashid, A.J., Josselyn, S.A., Yeomans, J.S., 2013. Cholinergic control of morphine-induced locomotion in rostromedial tegmental nucleus versus ventral tegmental area sites. *Eur. J. Neurosci.* 38, 2774–2785.
- Watt, M.L., Rorick-Kehn, L., Shaw, D.B., Knitowski, K.M., Quets, A.T., Chesterfield, A.K., McKinzie, D.L., Felder, C.C., 2013. The muscarinic acetylcholine receptor agonist BuTAC mediates antipsychotic-like effects via the M4 subtype. *Neuropsychopharmacology* 38, 2717–2726.
- Weiner, D.M., Levey, A.I., Brann, M.R., 1990. Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. *Proc. Natl. Acad. Sci. U. S. A.* 87, 7050–7054 doi:VL - 87.
- Wess, J., 2012. Novel muscarinic receptor mutant mouse models. *Handb. Exp. Pharmacol.* 2008, 95–117.
- Wess, J., 2004. Muscarinic acetylcholine receptor knockout mice: novel phenotypes and clinical implications. *Annu. Rev. Pharmacol. Toxicol.* 44, 423–450.
- Wess, J., 2003. Novel insights into muscarinic acetylcholine receptor function using gene targeting technology. *Trends Pharmacol. Sci.* 24, 414–420.
- Wess, J., Duttaroy, A., Gomez, J., Zhang, W., Yamada, M., Felder, C.C., Bernardini, N., Reeh, P.W., 2003. Muscarinic receptor subtypes mediating central and peripheral antinociception studied with muscarinic receptor knockout mice: a review. *Life Sci.* 72, 2047–2054.

- Wess, J., Eglen, R.M., Gautam, D., 2007. Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. *Nat. Rev. Drug Discov.* 6, 721–733.
- Witkin, J.M., Catlow, J.T., Wishart, G.N., Heinz, B.A., 2014. M1 and M2 muscarinic receptor subtypes regulate antidepressant-like effects of the rapidly acting antidepressant scopolamine. *J. Pharmacol. Exp. Ther.* 351, 448–456.
- Woolley, M.L., Carter, H.J., Gartlon, J.E., Watson, J.M., Dawson, L.A., 2009. Attenuation of amphetamine-induced activity by the non-selective muscarinic receptor agonist, xanomeline, is absent in muscarinic M4 receptor knockout mice and attenuated in muscarinic M1 receptor knockout mice. *Eur. J. Pharmacol.* 603, 147–149.
- Yamada, M., Lamping, K.G., Duttaroy, A., Zhang, W., Cui, Y., Bymaster, F.P., McKinzie, D.L., Felder, C.C., Deng, C.X., Faraci, F.M., Wess, J., 2001. Cholinergic dilation of cerebral blood vessels is abolished in M(5) muscarinic acetylcholine receptor knockout mice. *Proc. Natl. Acad. Sci. U.S.A.* 98, 14096–14101.
- Yamasaki, M., Matsui, M., Watanabe, M., 2010. Preferential localization of muscarinic M1 receptor on dendritic shaft and spine of cortical pyramidal cells and its anatomical evidence for volume transmission. *J. Neurosci.* 30, 4408–4418.
- Yan, Z., Flores-Hernandez, J., Surmeier, D.J., 2001. Coordinated expression of muscarinic receptor messenger RNAs in striatal medium spiny neurons. *Neuroscience* 103, 1017–1024.
- Yan, Z., Surmeier, D.J., 1996. Muscarinic (m2/m4) receptors reduce N- and P-type Ca^{2+} currents in rat neostriatal cholinergic interneurons through g-protein pathway. *J. Neurosci.* 16, 2592–2604.
- Yi, F., Ball, J., Stoll, K.E., Satpute, V.C., Mitchell, S.M., Pauli, J.L., Holloway, B.B., Johnston, A.D., Nathanson, N.M., Deisseroth, K., Gerber, D.J., Tonegawa, S., Lawrence, J.J., 2014. Direct excitation of parvalbumin-positive interneurons by M1 muscarinic acetylcholine receptors: roles in cellular excitability, inhibitory transmission and cognition. *J. Physiol.* 592, 3463–3494.
- Young, M.B., Thomas, S.A., 2014. M1-Muscarinic receptors promote fear memory consolidation via phospholipase C and the m-current. *J. Neurosci.* 34, 1570–1578.
- Zhang, H.M., Zhou, H.Y., Chen, S.R., Gautam, D., Wess, J., Pan, H.L., 2007. Control of glycinergic input to spinal dorsal horn neurons by distinct muscarinic receptor subtypes revealed using knockout mice. *J. Pharmacol. Exp. Ther.* 323, 963–971.
- Zhang, W., Basile, A.S., Gomeza, J., Volpicelli, L.A., Levey, A.I., Wess, J., 2002a. Characterization of central inhibitory muscarinic autoreceptors by the use of muscarinic acetylcholine receptor knock-out mice. *J. Neurosci.* 22, 1709–1717.
- Zhang, W., Yamada, M., Gomeza, J., Basile, A.S., Wess, J., 2002b. Multiple muscarinic acetylcholine receptor subtypes modulate striatal dopamine release, as studied with M1–M5 muscarinic receptor knock-out mice. *J. Neurosci.* 22, 6347–6352.
- Zhang, Y., Dyck, R.H., Hamilton, S.E., Nathanson, N.M., Yan, J., 2005. Disrupted tonotopy of the auditory cortex in mice lacking M1 muscarinic acetylcholine receptor. *Hear. Res.* 201, 145–155.
- Zhang, Y., Hamilton, S.E., Nathanson, N.M., Yan, J., 2006. Decreased input-specific plasticity of the auditory cortex in mice lacking M1 muscarinic acetylcholine receptors. *Cereb. Cortex* 16, 1258–1265.
- Zheng, F., Seeger, T., Nixdorf-Bergweiler, B.E., Alzheimer, C., 2011. Layer-specific processing of excitatory signals in CA1 interneurons depends on postsynaptic M2 muscarinic receptors. *Neurosci. Lett.* 494, 217–221.
- Zheng, F., Wess, J., Alzheimer, C., 2012. M2 muscarinic acetylcholine receptors regulate long-term potentiation at hippocampal CA3 pyramidal cell synapses in an input-specific fashion. *J. Neurophysiol.* 108, 91–100.
- Ztaou, S., Maurice, N., Camon, J., Guiraudie-Capraz, G., Kerkerian-Le Goff, L., Beurrier, C., Liberge, M., Amalric, M., 2016. Involvement of striatal cholinergic interneurons and M1 and M4 muscarinic receptors in motor symptoms of Parkinson's disease. *J. Neurosci.* 36, 9161–9172.