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α9-nAChR knockout mice exhibit dysregulation of stress responses, affect and reward-related behaviour

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ABSTRACT

The α9α10-subtype of nicotinic acetylcholine receptor (nAChR) has recently garnered interest in biomedicine and is being pursued as an analgesic target. However, the receptor exhibits diverse tissue distribution, the function of which is known to varying degrees, and targeting this receptor for clinical treatments without a broad understanding of its function may have adverse consequences. The α9α10-nAChR is expressed in the adrenal and pituitary glands, suggesting a potential role in the stress response, but little is known about its function in this tissue. Here we determined a role for the α9α10-nAChR in behavioural and physiological stress responses, by comparing the stress- and affect-related phenotypes of wildtype and α9-nAChR knockout mice. Naïve knockout mice exhibited largely normal behaviour on standard tests of affective behaviour. However, after sub-chronic restraint stress knockout mice showed significantly decreased stress-induced arousal and increased anxiety-like behaviour when compared to wildtype animals. Physiologically, corticosterone responses were muted in knockout mice after an acute stressor, but exaggerated in response to the same stressor after undergoing sub-chronic stress. Behavioural profiling of the α9-nAChR knockout mice in the home-cage revealed that circadian patterns of activity were altered when compared to wildtype controls. Furthermore, knockout mice showed altered responses to a period of reward discounting, resulting in anhedonia-like behaviour in a sucrose preference test where WT mice continued to seek reward. These experiments uncover a novel role for the α9α10-nAChR in mounting a normal stress response and in the regulation of affective- and reward-related behaviour, and suggest that pursuing the receptor for clinical treatments may not be as straightforward as has been suggested.

1. Introduction

The α9α10 subtype of nicotinic acetylcholine receptors (nAChRs) has become of increasing interest in biomedicine, particularly in pain research. The receptor is currently being pursued as a novel target for analgesic drugs (see [1] for review), however, functional expression of α9α10-nAChR protein in pain-relevant tissues (peripheral nerves and central nervous system) has not been found [2,3]. Instead, the tissue distribution of α9α10-nAChRs extends to many other sensory, immune and endocrine tissues including sensory epithelia [2,4,5], breast and lung epithelia [6–9], bone [5], keratinocytes [10,11], immune cells [12,13], and endocrine cells [2,14]. The functional significance of receptor expression in this large range of sites has been studied to varying degrees. The expression of α9α10-nAChRs in endocrine cells, namely pituitary [2] and adrenal tissue [14], implicates a role in the hypothalamic-adrenal-pituitary (HPA) axis and sympatho-adrenergic system and therefore a role in the stress response. However, no functional studies of this receptor have yet been performed in relation to stress. Given the psychological dimension to pain, it is possible that any pain modulation by α9α10-nAChRs occurs via an indirect influence on the sensory domain.

It is well recognised that systems of pain, stress, affect and reward interact [15–21]. The HPA-axis and sympatho-adrenergic system are involved in some of the most common chronic pain disorders including fibromyalgia, chronic lower back pain and rheumatoid arthritis [22,23]. The high rate of comorbidity of chronic pain and affective disorders has been mirrored in some, though not all [24], animal studies showing chronic stress-induced hyperalgesia [21,25–27] and chronic pain-induced depression- [28] and anxiety-like behaviour [29]. Chronic pain is also associated with changes, and often dysfunction, in

Abbreviations: CORT, corticosterone; DSA, drinking session adaptation; EPM, elevated plus maze; FA, free adaptation; KO, knockout; LHS, left hand side; nAChR, nicotinic acetylcholine receptor; NPA, Nosepoke adaptation; RFID, radiofrequency identification; RHS, right hand side; SD, sucrose discounting; SPT, sucrose preference test; WT, wildtype

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neural circuits underlying reward and motivation (see [18] for review). The mechanisms of these complex interacting systems have not been fully characterised, but nicotinic influences in pain [30–32], stress and affect [33–42] and reward [43–45] are known. A number of nAChR subunits have been implicated in these processes (see below), but the α9α10-nAChR subtype has not yet been considered.

The specific nicotinic receptor subtypes involved in cholinergic control of both mood and pain are not definitively known, as many studies have relied on pharmacological manipulations that lack specificity and selectivity (e.g. see [46,47]). Pharmacological tools have their limitations in characterising the functional roles of receptors in a whole organism, as selectivity cannot always be guaranteed. This has been demonstrated by studies of analgesic α9-nAChR-inhibitors [48,49] that were subsequently discovered to have alternative targets [50–52], which may have misguided the utility of α9-nAChR inhibitors for analgesia. The use of genetic manipulation in animals has allowed for a greater insight into the precise roles of nAChR subunits, and has, for example, singled out β2- and α7-containing nAChRs as important for nicotinic antidepressant effects [34,37–39] and α4- and β2-subunits for anxiety-like behaviour [33,36,40]. The β2 subunit has also been found to play a role in depression in human studies [53]. Similarly, transgenic mouse studies have recognised α4 [30], α5 [54], α6 [55] and α7 [56] subunits as key subunits for nicotinic analgesia and α4 [45] and β2 [57] subunits for reward. No behavioural studies of α9-nAChR KO mice have yet looked at the role of this subunit in the interrelated systems of stress, affect and reward. While the global knockout of receptor subtypes does have limitations such as unknown compensation during development, the phenotypic changes observed in transgenic animals can be attributed to the specific target alone. Thus, to characterise the role of the α9α10-nAChR in the stress-response, affective regulation and reward seeking we utilised mice with a germline deletion of the α9-nAChR. The behavioural and physiological responses to acute and sub-chronic stress of α9-nAChR KO mice were compared with those of wildtype mice and an important role of the α9α10-nAChR in the regulation of stress responses and affective behaviour was uncovered. Results also showed a significant role of the α9α10-nAChR in regulating circadian activity and in reward-related affective behaviours in the home cage environment.

2. Materials and methods

All experiments involving animals were approved by the University of Sydney Animal Ethics Committee (AEC. Protocol number K00/12-2011/3/5650). Experiments were performed under the guidelines of the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council, Australia, 7th Edition). Great care was taken to minimise animal suffering during these experiments and to reduce the number of animals used.

2.1. Animals

10–12 week old male mice of wildtype (WT) 129/SvEv strain and α9-nAChR knockout (KO) on the 129/SvEv background were used. KO mice on the 129/SvEv background were originally obtained from Dr. Douglas Vetter (Tufts Univ, Boston MA) [58] at the F6 generation and were back-crossed a further two times to F8 generation. WT and KO lines were maintained via homozygote crosses, generated from heterozygote matings, ensuring matched backgrounds. Receptor deletion was confirmed by genotyping using standard PCR procedures. The co-expression of α9 and α10 nAChR subunits is required to form functional channels. The α10 subunit fails to reconstitute a functional receptor alone, or in combination with any other known nAChRs, except for α9 [3,59], and is known to confer functionality to the α9-nAChR. Thus, deletion of the α9 subunit effectively knocks out functional α9α10-nAChRs.

Mice were bred in-house and maintained on a standard 12-h light/dark cycle (lights on at 0600) in standard individually ventilated cages with *ad libitum* access to food and water. Bedding was changed once weekly and at least 3 days prior to stress-hormone testing so as not to confound experiment-induced stress with that resulting from cage cleaning [60]. No more than 6 animals were housed together in a single standard cage.

2.2. Stress paradigm

All stress testing was conducted during the daily light phase. To limit the influence of circadian factors, application of the final stressor and all blood collection was undertaken between 0900 and 1100 h. Control animals remained in the holding room and housed 2 per cage so that blood collection could be completed within 1–2 min of cage disturbance [61,62]. Stressed animals were housed separately from control animals to avoid chemosignalling of emotional information from stressed to non-stressed conspecifics [63,64].

Stress-induced alterations in behaviours thought to be indicative of affective state were compared between the WT and KO genotypes. Behavioural tests were followed by measurement of physiological stress responses, assessed by circulating corticosterone levels (see Section 2.6). Animals underwent either acute (forced swim test only) or sub-chronic (restraint, elevated plus maze, forced swim test) stress [65,66]. Sub-chronic stressors were applied at varying times of day to avoid habituation; acute stress was applied at the same time of day for all groups (Table 1.). Since stress-induced changes in α9-nAChR expression have been shown to occur within 5 days [14], a sub-chronic stress (~1 week) paradigm was used, rather than chronic stress (≥4 weeks; [67]). This shorter stress period ensured that animal suffering was minimised and habituation to the stressors was avoided [68], while allowing enough time for potential α9-nAChR-dependent changes to take place.

Numbers indicated are per group for each genotype. “CORT” is the blood collection for the measurement of plasma corticosterone.

Table 1
Timing schedule of stress paradigm.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Acute Stress (n = 8)						FST, CORT 09:00–11:00
Sub-chronic Stress (n = 7–8)	Restraint stress 19:00–21:00	12:00–14:00	07:00–09:00	15:00–17:00	EPM 11:15–14:00	FST, CORT 09:00–11:00
Naïve EPM (n = 10–12)					EPM 11:15–14:00	
Naïve CORT (n = 12)						CORT 09:00–11:00